Research Paper

Catha edulis Forsk (khat) reduces spontaneous and rewarded alternation in female mice

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ABSTRACT

Around 10 million people chew the fresh leaves and twigs of Catha edulis Forsk (khat), which synthesize cathinone, for its psychostimulatory effect. Several studies have reported that regular khat users show executive and cognitive dysfunction, such as impaired inhibitory control and poor performance on memory tests. In this study, the effect of fresh khat extract (100 and 250 mg/kg) on spatial working memory and short-term memory in mice was assessed using spontaneous and rewarded alternation T-maze tests. In the spontaneous alternation test, mice treated with fresh khat extract decreased their spontaneous alternation level to around chance level, and it remained at this level over the 7 days of khat administration and testing. On testing after a 7-day khat free period, the previously khat treated mice showed alternation level above chance but below their pre-khat alternation level. In the rewarded alternation test, acute treatment with khat caused the mice to alternate well below chance level, and then over the next 3 days of khat treatment, the alternation level increased. After a 2 and 9-day khat-free period, the previously khat treated mice alternated above chance level but below their pre-khat level. In both these tests, the mice did not show any position preference before khat treatment; however, during the khat treatment, the mice showed a right side tendency. The results show that khat treatment causes persistent changes in alternation behavior and promotes perseverative behavior, presumably due to its effect on the neural circuits activity and the neurotransmitters and promotion of position preference.

1. Introduction

In southwestern parts of the Arabian Peninsula and along eastern Africa, people chew the fresh young leaves and twigs of the shrub, Catha edulis Forsk, commonly called khat, for its psychostimulatory effects (Magdum, 2011; Patel, 2015). This practice is centuries old and deeply integrated into these societies and communities’ traditional and social activity (Bedada and Engidawork, 2009; Mohammed et al., 2014). An estimated 10–20 million people are regular khat users (Corkery et al., 2011; Bogale et al., 2016), with the age of starting as low as 12 years (Al-Mugahed, 2008). Cathinone is the principal psychoactive agent in khat and an intermediate metabolite in the plant’s biosynthetic pathway (Krizevski et al., 2007). It was isolated and identified in 1975 (Szendr, 1980). It is a phenylethylamine structurally related to amphetamine and producing similar psychostimulatory effects (Al-hebshi and Skaug, 2005; Agee, 2008). There are two other less active psychoactive compounds in khat: cathine (norpseudoephedrine) and norephedrine; both are the terminal products of the biosynthetic pathway involving cathinone (Krizevski et al., 2007). Similar to amphetamine, khat produces a feeling of euphoria, energy, alertness, and confidence, as well as loquacity, appetite suppression, and insomnia (Balint et al., 2009). It also produces sympathomimetic effects such as hypertension, tachycardia, and hyperthermia, to which tolerance develops with regular use (Nabuzoka and Badhadhe, 2000).

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Though some users, especially students, consider that khat use assists in studying and learning (Ayano et al., 2019), regular khat use can lead to impaired executive and cognitive function. Performance is lower for visual memory, perception, perceptual speed, and long-term memory (Khattab and Amer, 1995); inhibitory control is impaired (Colzato et al., 2011a), and there are deficits in working memory and cognitive flexibility (Colzato et al., 2011b; Hoffman and Al Absi, 2013). Berihu et al. (2017) concluded from a meta-analysis of human and rodent studies that khat can affect short term memory but not long-term memory, and Mohammed et al. (2014) found in mice, depending on the pattern of khat administration, effects on short term memory in a multiple T-maze. Since first described by Tolman in 1925, spontaneous alternation...
behavior (SAB) in T- or Y-maze has been used widely as a simple test to assess cognitive function (Richman et al., 1987; Lalonde, 2002). SAB is seen when the animal, after visiting one of the arms of the maze in the first run of the trial, visits another not recently visited arm in the second run of the trial. A variation of this test is the rewarded alternation test. In this test, the animal is trained to visit the arm that contains a food reward and then, on the next visit, to go to the other arm, which is now baited with a food reward (Deacon and Rawlins, 2006). SAB tests the working memory of the animal, i.e., memory utilized when the response is based only on the information within a trial and not with reference to previous trials (Lalonde, 2002), the latter being a test of reference memory. In this study, we tested whether fresh khat would affect spatial working memory or short-term memory in mice using the spontaneous and rewarded alternation tests in a T-maze.

2. Material and methods

2.1. Experimental animals

Adult female Swiss albino mice (7–8 weeks, 16–22 g), obtained from the School of Biological Sciences, University of Nairobi, were group-housed (5 per cage) under a 12:12 h light/dark cycle, ambient room temperature (22–23 °C), with mouse food pellets (Unga Group Ltd®, Kenya) and water available ad libitum. Every three days, the cages were cleaned, and the wood shaving bedding replaced. The mice were habituated to handling and the test room for 5 days before the start of the experiments. A total of 60 mice were used: 30 for the spontaneous alternation test and 30 for the rewarded alternation test. For each test, the mice were divided into 3 treatment groups (n = 10): (1) saline, (2) 100 mg/kg, and (3) 250 mg/kg fresh khat extract, and weights were measured weekly. The khat doses were selected by estimating the amount of khat that would normally be consumed in a single khat session, which is between 200–500 g. We assume that the chewer consumes the exudate and discards the chewed leaves and twigs. So, with yield of around 3.5 %, the amount consumed by the user, assuming to be an adult male with an average weight of 70 kg, the doses would be (200 g × 3.5 %)/70 kg = 100 mg/kg and 250 mg/kg for the higher amounts of khat consumed.

Female mice were used in this study to prevent the aggressive behavior that can occur within group housed male mice, unlike with male rats. This would result in difficulties in statistical analysis using repeated measures ANOVA where group size changes could occur during the experiment due to the aggressive behavior of male mice towards their cage mates and, therefore, incomplete data collection. Furthermore, the prevalent concern of female estrus cycling affecting the results, and therefore the preference of male subjects in neuroscience experiments, has been shown in most cases not to affect the variability of the results (Becker et al., 2016; Berry, 2018).

The Guide for the Care and the Use of Laboratory Animals (National Academies of Science, USA, 2011) was followed. Ethical approval was granted by the Biosafety, Animal Use and Ethics Committee, Department of Veterinary Anatomy and Physiology, University of Nairobi (Ref: FVM BAUEC/2018/174).

2.2. Khat extract preparation

Fresh khat (800 g) was purchased from the Westlands market, Nairobi, Kenya, and transported in an icebox, and stored at −20 °C until used. A specimen was deposited with and identified by the University of Nairobi herbarium (voucher specimen CKM2017/06). The leaves and bark of the fresh khat were cut into small pieces and homogenized in distilled water (100 gm/500 ml). The homogenate was coarsely filtered, finely filtered, and centrifuged. The supernatant was then decanted and lyophilized (Christ Alpha 1–6, Medizinischer Apparatebau®, Germany) and stored in a desiccator at −4 °C until used. The yield was 3.30 %.

2.3. T-maze spontaneous alternation

The T-maze was made from chipboard, painted grey with a 30 × 10 × 20 cm start alley, and two 30 × 10 × 20 cm goal (side) arms with a central partition at the T junction. The side arms could be closed by guillotine doors. Individual mice were placed at the base of the start arm of the T-maze and allowed free access to either goal arm. When the mouse entered one of the goal arms, the guillotine door on that arm was lowered, and the mouse was confined to that arm for 30 s. Following this confinement period, the mouse was taken out and returned to the start arm and again allowed access to either goal arms. If the mouse chose the arm it had not chosen in the first run; this was recorded as a correct response (C), i.e., it had alternated between the two goal arms. If the mouse chose the same arm it had chosen in the first run; this was recorded as an incorrect choice (I). If the mouse did not enter either arm in 90 s (trial time), it was removed from the maze and recorded as a failed run (F). After its second run, the mouse was removed from the maze and placed in the holding container for 30 s, the intratrial interval. After each trial, the apparatus was cleaned with a dry tissue to remove the droppings and wiped with a moist cloth. Ten trials per mouse per day were done. On days 1–3, the mice were tested in the T-maze without any treatment. On days 4–10, 15 min prior to testing, the mice were injected intraperitoneally with saline or khat extract (injection volume 0.1 ml) according to their groups and tested in the T-maze. On days 11–17, the mice were kept in their home cages without any treatment or testing. After this khat free period, on days 18–20, the mice were tested again for the spontaneous alternation but without khat treatment.

2.4. Rewarded alternation

For rewarded alternation, for 5 days, the mice were habituated to handling and to a breakfast cereal food (Choco Rice, Morning Harvest®, Nairobi, Kenya) in their home cage to eliminate hyponeophagia. The mice were then trained to alternate to a food reward in the T-maze for 4 days. On the 1st day of training, the doors of the T-maze goal arms were kept open and the food wells located at the end of each goal arm filled with the food reward. The mice from a single home cage, food-deprived overnight, were placed in the start arm of the T-maze and allowed to explore the maze for 3 min and allowed to consume the food reward. This was repeated 4 times for each home cage. On the 2nd day of training, both arms of the T-maze were baited with the food reward. One arm was designated the sample arm and the other the choice arm that was closed during the first run of the trial. The mouse was placed in the start arm and allowed to run into the open sample arm and consume all the food reward. Once the mouse had consumed all the food reward, it was taken out, and with both the sample and choice arms open, returned to the start arm and allowed to choose either arm. If the mouse chose the choice arm (correct choice C), it was given time to consume the reward. If it did not choose the choice arm but instead went back to the original sample arm, an incorrect arm choice had been made (I), and the mouse was confined to that arm for the time it would have taken it to consume the reward, i.e., 30 s. If the mouse did not run or ran back and forth in the start arm without choosing any arm, it was picked up from the maze and placed in the holding container for 30 s before the next trial. Ten trials per mouse per day were done, with the sampling arm alternated for each trial, i.e., 5 right and 5 left. This training was repeated on the 3rd and 4th day of the training session.

After the training session, the baseline measurement with no khat treatment (Day 0) was done. For this, both arms of the T-maze were baited with the food reward and kept open. The mouse was placed in the start arm and allowed to choose either arm and allowed time to consume the food reward in that arm. Once it had consumed all the reward in that arm, it was taken out and returned to the start arm and allowed to choose either arm. If the mouse chose the arm it had not chosen in the first run; it was given time to consume the reward. If instead, it went to the arm it had visited on the first run, the wrong arm choice had been...
made, and it was confined to that arm for the time it would have taken it to consume the reward. Following the baseline measurement, on day 1, the mice depending on their group, were injected ip either with saline or 100 mg/kg or 250 mg/kg fresh khat extract (injection volume 0.1 ml), 15 min before the start of their trial session which consisted of 5 trials per day over the next 4 days. After this, the mice were kept in their home cages without any treatment and tested in a khat-free state on days 6 and 13.

2.5. Position preference

It has been shown that rats and mice of different strain and gender show differences in position preference, also referred to as side or lateral preference, and this can influence the result of T-maze alternation (Andrade et al., 2001). In particular, position preference can worsen the alternation levels as the subject prefers to turn at the T-maze junction to its preferred side, be it right or left. In order to examine if position preference could have an influence on the T-maze alternation, we calculated a position preference rate based on the formula given by Wu et al. (2018).

(The number of preferred side the subject has chosen / total runs performed) * 100

As per Wu et al. (2018), we selected the left side as the preferred side. A position performance rate of 50 % shows no position preference, while a rate of less than 50 % shows a preference for the right side and above 50 % preference for the left side.

2.6. Data analysis

The data are expressed as mean percentage ± sem. A two-way repeated-measures Analysis of Variance (ANOVA, treatment vs day) followed by Dunnett’s multiple comparison post-hoc test was performed using GraphPad Prism (v 8.0, GraphPad Software, San Diego, CA). χ² analysis was done out to determine if the total group alternation differed from chance. The significance level was set at p < 0.05.

3. Results

3.1. Spontaneous alternation

3.1.1. Mice weight

Saline-treated mice steadily increased their weight to 22.5 % by the end of the experiment period. During the khat treatment period, the 100 mg/kg group showed a decrease of 9.4 % (week 1) and 7.8 % (week 2). In the 250 mg/kg group, their weight decreased by 9.4 % (week 1) and 7.4 % (week 2). Both groups rapidly regained their weight to the pre-treatment level after cessation of khat administration.

3.1.2. Spontaneous alternation

The spontaneous alternation percentage is shown in Fig. 1a. Two-way repeated measures ANOVA showed a significant effect of treatment (F (2,27) = 45.45, p < 0.0001) and days (F (5,133,143.9) = 17.59, p < 0.0001) and interaction between treatment and days (F (24,324) = 8.205, (p < 0.0001). Dunnett’s multiple comparison test showed that on days 1–3, with no khat treatment, the alternation level between the groups was not significantly different. On days 4–10, the treatment days, saline-treated mice increased their alteration level up to day 5, after which it decreased although it remained above chance level, i.e., above 50 %, and significantly higher than the khat treated groups (Fig. 1a). On the first day of khat treatment (day 4), both the 100 and 250 mg/kg khat treated mice decreased their alternation levels to around 50 %, and over the next 3 days, further reduced their alternation levels to below 50 %. Following the 7-day khat free period, testing on days 18–20, without any treatment, the previously saline-treated group alternated at the same level as before, whilst the previously khat treated groups alternated around the chance level.

To determine whether the alternation scores differed significantly from chance level, χ² analyses were carried out for the total group alternation scores. The saline-treated group alternated significantly above chance over all the experimental days. In the 100 and 250 mg/kg khat treated mice, on the 1st and 2nd day of khat treatment (day 4 and 5), the alternation level dropped to chance levels (for 100 and 250 mg /kg khat treated groups on day 4: χ² = 1 and 0.04, df = 1; and on day 5: χ² = 1.4 and 2.6, df = 1). Following khat treatment on the subsequent 7 days, the 100 mg/kg khat group alternated significantly below the chance level on day 7 and 8 and then at the chance level on day 9-10. The 250 mg/kg khat group alternated significantly below chance level over days 6-10. After the 7-day khat-free period, testing on day 18–19, both the previously 100 and 250 mg/kg khat treated groups alternated at chance level.

3.1.3. Position preference

The position preference rate is shown in Fig. 1b. Before khat administration (days 1–3), the position preference rate for the saline group had a range of 47.0–56.5 %. The 100 mg/kg group had a range of 42.5–55 %, and the 250 mg/kg had a range of 49.0–50.5 % . The saline group position preference rate oscillated around 50 % for the duration of the experiment. For 100 mg/kg group during khat treatment (days 4–10), the rate decreased to 33.5 %, and in the 250 mg/kg group, the rate decreased to 37 %. After the khat free period, the preference rate in the 100 mg/kg group increased to 47.5 %, while in 250 mg/kg group, the rate increased to 45 % on day 19 and decreased to 40 % on day 20.
3.2. Rewarded alternation

3.2.1. Weight

Saline treated mice increased their weight by 18 % over the course of the experiment. The 100 mg/kg group reduced their weight by 2.9 %, and the 250 mg/kg group reduced their weight by 5.7 %, and both groups regained their weight when the khat treatment was stopped.

3.2.2. Rewarded alternation

The percentage rewarded alternation level is shown in Fig. 2a. Two-way repeated measures ANOVA analysis showed a significant effect of treatment ($F_{(2,27)} = 21.90, p < 0.0001$), days ($F_{(12,162)} = 16.90, p < 0.0001$), and interaction ($F_{(12,162)} = 5.34, p < 0.0001$) between the main effects. After the 4-day training phase, on test day 0 (no treatment), all 3 groups alternated around 80 %. On the treatment days, the saline treated group alternated around this level, and Dunnett’s multiple comparison test showed that this alternation level was significantly higher than the 100 and 250 mg/kg khat treated groups. The 100 mg/kg khat group showed alternation levels below 50 % on day 1–3, and above 50 % on day 4, the last treatment day. After a 2-day and 9-day khat free period, and tested in a khat-free state, this group showed an alternation level above 50 % on test day 6 and 13. The 250 mg/kg group showed alternation well below 50 % on all days of khat treatment (days 1–4). On the first day of khat treatment, this group’s alternation level was 4 %, and this was primarily due to only 20 % of the mice in this group displaying alternation behavior. On the subsequent days, the percentage of mice in this group showing alternation behavior increased to 60 %, with the mean alternation level rising to 24 %. On testing in a khat-free state after the 2-day khat free period (day 6), this group showed an alternation level around 50 %, and after 9 days of khat free period (day 13), the alternation level was above 50 % with all the mice now showing 100 % alternation behavior on both these days.

Chi-squared analyses of the total group alternation scores showed that the saline-treated mice alternated significantly above the chance level on all days. The 100 mg/kg group alternation did not differ from the chance level on day 2–4, the khat treatment days, but was significantly above the chance level on days 6 and 13, i.e., testing following a 2-day and 9-day khat free period. For the 250 mg/kg group, alternation levels were significantly below chance on all khat treatment days though the alternation level increased over the days of khat treatment. When tested in a khat-free state on days 6 and 13, this group alternated at chance level.

3.2.3. Position preference

The position preference rate is shown in Fig. 2b. Before khat treatment, the position preference rates for saline, 100 mg/kg, and 250 mg/kg groups were 47.8 %, 51 %, and 49 %, respectively. The rate for the saline treated group oscillated around 50 % over the days of the experiment. For the 100 mg/kg and 250 mg/kg groups, on day 1 of khat treatment, adjusting for mice that failed to show alternation, the preference rate was 38.5 % and 20 %, respectively. By day 4, the last day of khat treatment, the rate was 53.6 % for 100 mg/kg group and 45.4 % for the 250 mg/kg group. After the khat free period, the preference rate on day 6 and 13 ranged between 45.6–49.5 % for all the groups.

4. Discussion

In this study, we tested the effect of khat on spatial working memory or short-term memory in mice using the spontaneous and rewarded alternation test in a T-maze. In both spontaneous and rewarded alternation tests, under the influence of khat, the mice alternated at significantly lower levels than saline-treated mice, and these alternation levels were dose-dependent. $\chi^2$ analyses of the alternation level in both spontaneous and rewarded alternation showed that khat treated mice either alternated at chance or significantly below chance level, thus displaying perseverative behavior.

The acute effect of khat (the first day of khat treatment) on spontaneous and rewarded alternation was different. In the spontaneous alternation test, both khat treated groups decreased alternation to chance level. This acute effect by a psychostimulant in causing the alternation level to decrease to chance level is also seen with low dose amphetamine treatment, while with higher amphetamine dose, perseverative behavior is observed (Kokkinidis and Anisman, 1976a, b; Bruto and Anisman, 1983). Treatment with scopolamine, an anticholinergic, also causes alternation to reduce chance level (Kokkinidis and Anisman, 1976a).

In the rewarded alternation test, on the first day of treatment (acute effect), both the khat treated groups showed alternation significantly below chance level, i.e., they displayed perseverative behavior. Furthermore, the degree of alternation was more much lower in the high khat dose group than in the low khat dose group, i.e., the high khat dose enhanced perseverative behavior. The reason for the difference in alternation level between the spontaneous and rewarded alternation tests is not clear. A similar enhancement of perseverative behavior was reported for amphetamine treated mice that were pre-exposed to the maze before testing (Kokkinidis and Anisman, 1976a, b; Bruto et al., 1983). As before the rewarded alternation test in our study, the mice underwent several days of training in the T-maze; this pre-exposure to the apparatus may be the cause of the increase in perseverative behavior seen on the first day of khat treatment.

The behavioral response to repeated khat treatment between the spontaneous and rewarded alternation tests also differed. In the spontaneous alternation test, daily khat treatment over 7 days, caused in the low dose khat treated mice, the alternation level first to further decrease and then to increase to chance level by the last 2 days of treatment. In
the high khat dose-treated mice, the alternation levels dipped below chance level by the third day of khat treatment, reflecting perseverative behavior. This result is different from that seen with repeated amphetamine treatment, which caused attenuation of perseverative behavior (Kokinidis and Anisman, 1978a,b; Bruto and Anisman, 1983; Bruto et al., 1983). However, in contrast to spontaneous alternation, in rewarded alternation, in both khat treated groups, the extent of perseverative behavior decreased with repeated khat treatment. In the low dose khat group, alternation increased to chance level, a response also seen with repeated amphetamine treatment (Bruto et al., 1983). The decrease in perseverative behavior with repeated khat treatment could be due to mice developing tolerance to khat; however, this seems unlikely as mice tested in a khat free state, after repeated khat treatment, alternated at a chance level rather than either at their pre-khat levels or the level of saline controls. This result has also been seen in subjects following chronic amphetamine treatment tested in a nondrug state. So, as with amphetamine, repeated khat treatment could reflect “...an inability [of the subjects] to filter environmental cues, or to attend/respond selectively to environmental cues...” (Bruto et al., 1983).

While cathinone is consumed predominantly by the traditional practice of chewing of fresh khat, the use of derivatives of cathinone, synthetic cathinones, has recently increased, and the effect of these substances on alternation behavior in the Y- and T-maze tests have been studied. In the Y-maze test, rats administered methylene, a substituted cathinone, in early and late adolescence showed when tested later in early adulthood impaired spatial memory (Daniel and Hughes, 2016); however, mice treated with a binge-like regimen of methylene showed no significant effect while mephedrone mice treated had reduced working memory in the T-maze test (Hollander den et al., 2013). Impaired performance in the Y-maze was found in male mice treated with a binge-like regimen of cathinone alpha-pyrrolidinopropiophenone (α-PPP) (Ray et al., 2019). The results of these maze studies differed depending on the synthetic cathinone studied, the dose, the pattern of administration, and the test used. Further studies will help to clarify the effect of these synthetic cathinones on cognitive function.

A factor that may have contributed to the response seen in mice in the high khat group was their considerable individual difference in reaction to khat. On the first day of khat treatment (acute), only 20% of the mice showed alternation. This number increased over the next 3 days of khat treatment with increasing alternation level. This individual difference was not seen in the low dose khat treated group in which all the mice showed alternation behavior. As this attenuation of perseverative behavior was seen only in the rewarded alternation test and not with the spontaneous alternation test where stable perseverative behavior was seen with repeated khat treatment, this difference in SAB may be induced by the method used.

The effect, if any, of the appetite suppression property of khat on the alternation level cannot be addressed by us at this stage as there are no studies done that have expressly studied this relationship. On the other hand, position preference has been suggested to worsen T-maze alternation results (Andrade et al., 2001). McFarland (1989) reported that d-amphetamine reduced SAB and increased the bias to turn in the same direction in a Y-maze.

Also, amphetamine induced rotational bias depends on the gender and the strain of rats (Storrie-Baker et al., 1992). In our calculation of the position preference rate, we found that before the khat treatment, the mice did not show a position preference. However, with khat treatment, there was a clear tendency of the mice to show right-side preference, and this tendency mirrored the decrease in the alternation levels recorded. As with changes in alternation level seen in the rewarded alternation test, the right-side preference decreased with repeated khat treatment. This decrease suggests that khat could stimulate or enforce the inherent position or side preference mechanism, considered to the nigrostriatal dopamine asymmetry, leading to decreased alternation levels seen in our experiment.

Further studies are required to explain this difference in SAB seen with khat treated mice tested with these different variations of the T-maze alternation test. Additional experiments with male mice and with a broader range of khat doses also need to be done. Also, though SAB is used extensively to study the effects of neural lesions and pharmacological interventions on spatial working memory or short-term memory, the underlying neurobiology of the SAB phenomenon is not clear (Lalonde, 2002), and interpretation of SAB remains a bit of a puzzle (Hughes, 2004).

Our results show that acute and chronic khat treatment in mice causes alternation levels to decrease to chance or below chance level, indicating that spatial working memory or short memory is impaired as this memory is needed to remember recently visited arms of the maze. Furthermore, as the alternation levels in the previously khat treated mice did not reach the level seen before khat treatment, exposure to khat may cause persistent changes in the neural circuits and neurotransmitters underlying position preference and short-term memory function.

Author contributions

CM collected the data and drafted the manuscript. JK and NP conceived and supervised the work. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors report no declarations of interest.

Ethical statement

The Guide for the Care and the Use of Laboratory Animals (National Academies of Science, USA, 2011) was followed. Ethical approval was granted by the Biosafety, Animal Use and Ethics Committee, Department of Veterinary Anatomy and Physiology, University of Nairobi (Ref: FVM BAUEC/2018/174).

CRediT authorship contribution statement

Caroline K. Murithi: Data curation, Formal analysis, Methodology, Investigation, Visualization, Writing - original draft, Writing - review & editing. Jacques M. Kabaru: Conceptualization, Methodology, Supervision, Formal analysis, Writing - review & editing. Nilesh B. Patel: Conceptualization, Methodology, Formal analysis, Supervision, Visualization, Writing - review & editing.

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