International Journal Dental and Medical Sciences Research
Volume 4, Issue 6, Nov-Dec 2022 pp 790-794 www.ijdmsrjournal.com ISSN: 2582-6018

Effect of Ginger (Zingiber Officinale) and Marijuana (Cannabis Sativa) On Fear and Anxiety in Swiss Mice.

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Submitted: 15-12-2022 Accepted: 26-12-2022

ABSTRACT
Aim: The effects of consumption of powdered marijuana diet and ginger diet on fear and anxiety were studied using Swiss mice.
Design: The mice were randomly assigned into three groups. Group 1 served as control and was fed with normal rodent chow. Group 2 was fed with ginger diet (5g of powdered ginger mixed with 95g of rodent chow, making 5% of ginger diet) while group 3 was fed with marijuana diet (5g of powdered marijuana mixed with 95g of rodent chow, making 5% of marijuana diet)
Methodology: Feeding was allowed for 14 days before the experiment started. The animals had free access to their feeds and water. Food and water intake were measured daily. The Elevated Plus-Maze was used to assess fear and anxiety in the mice.
Results: Showed that 5g ginger diet had no significant effect on the animal behaviour in terms of time spent in the light and dark fields as well as the time spent in grooming while 5g marijuana diet significantly increased time spent in light and decreased time spent in dark and time spent grooming. These alterations in the marijuana group indicate a reduction in fear and anxiety.
Conclusion: Consumption of 5% marijuana diet suppressed fear and anxiety in Swiss mice while consumption of 5g ginger did not affect fear and anxiety in these animals.

I. INTRODUCTION

Marijuana, which can also be called cannabis, weed, pot, or dope—refers to the dried flowers, leaves, stems, and seeds of the cannabis plant. The cannabis plant contains more than 100 compounds (or cannabinoids). These compounds include tetrahydrocannabinol (THC), which is impairing or mind-altering, as well as other active compounds, such as cannabidiol (CBD). CBD is not impairing, meaning it does not cause a "high" (Lee CM, Neighbours C, Woods BA, 2007). Marijuana is the most commonly used addictive drug after tobacco and alcohol. Its use is widespread among young people (NSDUH 2018). In 2018, more than 11.8 million young adults used marijuana in the past year. New initiates to use of marijuana were higher than that for cigarettes but slightly less than those for alcohol. According to Monitoring the Future survey, rates of past year marijuana use among middle and high school students have remained steady, but the number of teens in 8th and 10th grades who say they use it daily has increased. With the growing popularity of vaping devices, teens have started vaping tetrahydrocannabinol (THC) which is the ingredient in marijuana that produces the high, with nearly 4% of 12th graders saying they vape THC daily (NSDUH, 2018; Canada Health, 2018). In addition, fewer number of young people believe that regular marijuana use is risky (Canada Health, 2018).

Smoking, chewing and eating marijuana mixed food with high level of THC is on the rise amongst the youths. Relaxation or tension reduction is commonly identified as a marijuana use motive (Lee CM, Neighbours C, Woods BA, 2007). On the other hand, anxiety and panic reactions are the most commonly noted negative acute effects of marijuana intoxication (Crippa JA, Zuardi AW, Martín-Santos R et al. 2009). All other things being equal, THC appears to decrease anxiety at lower doses and increase anxiety at higher doses. Ginger (Zingiber officinale) is a plant native to Asia. The ginger spice comes from the roots of the plant. It is used as a food flavouring and medicine. Ginger's root or rhizome is the part used as a spice or healing aid. Depending on the variety, the inside of the root can be yellow, red, or white. It's harvested by pulling the entire plant out of the soil, removing the leaves and cleaning the root. Ginger can be consumed fresh, dried and stored as a spice, or made into tablets, capsules, and liquid extracts. Although ginger tea may help to calm some symptoms of anxiety, such as nausea or an upset stomach. Whether the ingestion of ginger in powdered form will affect fear and anxiety has not been thoroughly investigated. Fear is an intensely unpleasant emotion in response to perceiving or recognizing a danger or threat

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(Öhman and Wiens, 2004). Fear causes physiological changes that may produce behavioural reactions such as mounting an aggressive response or fleeing the threat. Fear in human beings may occur in response to a certain stimulus occurring in the present, or in anticipation or expectation of a future threat perceived as a risk to oneself.

Anxiety is a feeling of fear, dread, and uneasiness. It might cause you to sweat, feel restless and tense, and have a rapid heartbeat. It can be a normal reaction to stress. The main function of fear and anxiety is to act as a signal of danger, threat, or motivational conflict, and to trigger appropriate adaptive responses (Öhman and Wiens, 2004). For some authors, fear and anxiety are undistinguishable, whereas others believe that they are distinct phenomena. This study is aimed at investigating the effects of consumption of marijuana and ginger on fear and anxiety in Swiss mice.

II. MATERIALS AND METHODS

Preparation of ginger diet

Rhizomes of Zingiber officinale was peeled removing its cover, washed to remove impurities and then sliced to smaller sizes and dried. The dried samples were then pulverized using an electric blender to obtain a fine powder. The ginger powder was then stored in air-tight rubber container. From this, the ginger diet containing 5g of ginger**:**95g feed was prepared. This produced a ginger diet containing 5% of ginger.

Preparation of marijuana (Cannabis sativa) diet

The flowers of Cannabis sativa were grinded and sieved to obtain a fine powder. The powder was stored in a container. From this, the marijuana diet containing 5g of marijuana**:**95g feed was prepared. This produced a marijuana diet containing 5% of marijuana.

Preparation of experimental animals and treatment

Adult Swiss mice were obtained from the animal house of the Faculty of Basic Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. They were allowed one week to acclamatize. After acclimatization, the animals were randomly divided into three groups of 15 rats each. Group one (control) received distilled water. Group two consumed ginger diet while group three consumed marijuana diet.

Each mouse in a study group was individually housed in a plastic cage (27cm × 20cm) with the cover of the cages perforated for adequate ventilation. The cages were kept at standard room temperature and humidity of 28 ± 2˚C and 40-70% and a 12 hour light and 12 hour dark cycle. All mice had access to their appropriate feed and water ad libitum .

Procedure

The Elevated Plus Maze was made of wood-block and consisted of four arms (50cm long x 10cm wide) fixed to a central platform (50cm x 50cm): two had high walls (closed-arms) and two had no border in place of walls (open-arms). The maze was elevated to a height of 50cm.

The mice were brought individually to the testing room 1 hour before the test was started. For testing, the mice were placed in the centre square of the elevated plus maze facing the open arm. Behaviour was recorded by a group of experimentalists for a period of 5 minutes using stop watches. The primary measurements were the number of entries and the time spent in each arm, stretch-attend postures and grooming.

Statistical analysis

Data was analysed using statistical package for social sciences (SPSS version 21). The significant differences between group means was tested using analysis of variance (ANOVA) and multiple group means were compared using the post hoc test. Differences were considered significant at $P<0.05$.

III. RESULTS

The results of the study are presented in tables 1-5.

Table 1: Time spent in light and dark arms of the elevated plus maze

Groups	Time in light	Relative change (%)	Time in dark	Relative change (%)
Control	34.18±5.05	0	266.83±4.67	0
Ginger	34.25±4.09	0.20	266.03±4.08	-0.30
Marijuana	72.25±10.73*	111.38	228.00±10.77*	-14.55

Values are expressed as mean±SEM. (*) indicates statistically significant ($p<0.05$) difference when compared to control.

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Table 2: Number of entries into light and dark arms of the elevated plus maze

Groups	Entry into light	Relative change (%)	Entry into dark	Relative change (%)
Control	3.25±0.79	0	4.25±0.79	0
Ginger	5.00±1.06	53.85	6.00±1.06	41.18
Marijuana	5.21±0.42	60.31	6.21±0.42	46.12

Values are expressed as mean±SEM.

Table 3: Time in the center square of the elevated plus maze

Groups	Time in center square	Relative change (%)
Control	10.92±2.40	0
Ginger	11.07±2.13	1.37
Marijuana	30.00±3.48*	174.73

Values are expressed as mean±SEM. (*) indicates statistically significant ($p<0.05$) difference when compared to control.

Table 4: Recorded number of grooming and time spent in grooming

Groups	Number of grooming	Relative change (%)	Time spent grooming (sec)	Relative change (%)
Control	3.08±0.42	0	15.58±3.38	0
Ginger	2.80±0.38	-9.09	14.00±2.50	-10.14
Marijuana	2.64±0.29	-14.29	10.64±1.73	-31.71

Values are expressed as mean±SEM. (*) indicates statistically significant ($p<0.05$) difference when compared to control.

Table 5: Number of attended stretch and time spent stretching

Groups	Number of attended stretch	Relative change (%)	Time spent during stretch (sec)	Relative change (%)
Control	20.50±3.19	0	31.75±4.31	0
Ginger	20.93±2.75	2.10	31.07±3.81	-2.14
Marijuana	14.21± 1.61	-30.68	18.21±2.11*	-42.65

Values are expressed as mean±SEM. (*) indicates statistically significant ($p<0.05$) difference when compared to control.

IV. DISCUSSION

The Elevated plus maze (EPM) consists of two open arms and two closed arms in the shape of a plus. The open arms are aversive to mice because they are open and the maze is elevated. The closed arms provide a sense of safety because they are enclosed like most tests of anxiety (the light/dark box and open field). This task exploits the conflict between the natural tendency of mice to explore novel areas and fear of open spaces (Walf and Frye, 2007).

In this study, the animals that consumed marijuana diet spent a significantly longer time in the light and significantly shorter time in the dark when they were compared to the control, indicating that, they were emboldened and their behavior undermined natural fear for open spaces. The animals that consumed ginger did not show any

significant reactions with regards to their preferences for open or dark spaces.

Behaviors such as open arm activity and time spent in center square are considered exploratory and a greater frequency of these measures shows a greater level of exploration. The animals in marijuana group also showed a tendency to explore the center square for a longer time (table 3) and less stretch time implying that they exhibited less fear compared to the ginger and control groups. Fear behaviors include, closed arm activity, stretch attend posture, grooming frequency and duration, greater number of these measures implies a greater level of emotionality or fear (Walf and Frye, 2007). Risk assessment behaviors such as head dips and stretch attend postures are indexes of levels of anxiety. The demonstration of anxiety in this study was done using the elevated plus maze. In the elevated plus maze, the duration of grooming was observed to be marginally lower in the marijuana diet fed mice. Grooming is a displacement response and it is associated with anxiety in rodents when they are introduced into a novel environment (Bruijnzeel etal. 2016). Another behavior that strongly correlates with anxiety is the closed arm duration in the elevated plus maze. This duration was found to be significantly lower in the marijuana diet fed mice compared to control and ginger fed diet. Fearful mice would normally spend more time in the closed arms of the elevated plus maze. This also shows that the marijuana diet fed mice showed decreased fear and anxiety compared to control. The grooming frequency which is a measure of fear and anxiety was lower for the marijuana than the control and ginger values. The open arms duration was also significantly higher than the control values. These show a lower level of anxiety and fear. Open arms duration and the frequency is a behavior which point to decreased anxiety. These behaviors correlate strongly, and the higher their value, the less the anxiety level. So marijuana consumption may be reducing anxiety in the animals. On the other hand, open arm avoidance by rodents in the elevated plus maze gives a measure of anxiety which was shown more in the control group than the ginger fed diet group and least in the marijuana fed diet group.

Fear and anxiety are basically controlled by neural circuitry involving the amygdala mostly and the hypothalamus. Other areas of the brain that may be involved in the control of fear and anxiety are the nuclei of the hypothalamus. Electrical stimulation of the amygdala for instance is associated with fear and feeling of terror in the animals [Alò etal, (2014) and Alves etal.,(2009)].

V. CONCLUSION

Consumption of 5g ginger diet mixed in 95g feed showed no significant effect on various parameters of fear and anxiety studied while 5g marijuana diet in equal amount of feed significantly altered these parameters to indicate a suppression of fear and anxiety.

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