JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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Long-term Administration of *Cannabis sativa* Impairs Learning and Memory in CD1 Mice

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The effects of long term administration of *Cannabis sativa* on learning and memory in CD1 mice was studied. Twenty seven mice (male and female) were used for the experiment. They were assigned randomly into three groups (control, low dose and high dose) n = 9. Group I (the control group) was given 10 mL kg\(^{-1}\) of normal saline. Group II (The low dose group) received 10 mg kg\(^{-1}\) day\(^{-1}\) of *Cannabis sativa*; Group III (high dose group) received 20 mg kg\(^{-1}\) day\(^{-1}\) *Cannabis sativa*. Oral route of administration was used for all groups for 28 days. All three groups were allowed free access to food and water. After 28 days of administration of *Cannabis sativa*, learning and memory was assessed using the Morrice Water Maze (MWM). Results obtained, the swim latency during the reversal training were longer in the low dose group (p<0.01) and high dose group (p<0.001) fed *Cannabis sativa* compared to their control. This is indicative of reduced learning ability for the Cannabis-treated dose groups. The duration of time spent in the Northeast (NE) quadrant (which had the escape platform during the acquisition training) showed that animals in the test groups (low and high dose) spent shorter time (p<0.05) in the NE quadrant compared to their control, showing reduced memory in the *Cannabis* treated groups. Conclusively, long-term administration of *Cannabis sativa* impaired visuospatial learning in CD1 mice; hence its liberal use should be discouraged.

**Key words:** *Cannabis sativa*, learning and memory, mice, morris water maze
INTRODUCTION

Pot, weed, grass, ganja and skunk are some of the common terms used to describe the dried leaves of Cannabis. Cannabis is a genus of flowering plant that includes three putative species; Cannabis sativa, Cannabis indica and Cannabis ruderalis (Elsohly, 2007).

Cannabis sativa is an annual herbaceous plant of the family Cannabaceae. It has long been used for religious and medicinal purposes and as a recreational drug (due to its psychoactive, or mind-altering effects). Some therapeutic uses of Cannabis indica include; treatment of spasticity, movement disorders, asthma and glaucoma, allergies, inflammatory and infectious diseases (Grottenhermen and Russo, 2002).

Beside it advantageous effects, Cannabis sativa has been documented in several studies to have a link with symptoms of schizophrenia (Hall and Solowij, 1998), reduce food intake documented in several studies to have a link with symptoms of infectious diseases (Grotenhermen and Russo, 2002).

Assessment of learning and memory: The Morris water maze was used to assess visuospatial learning as described by Vorhees and Williams (2006) and used by Bisong et al. (2008).

RESULTS

Comparison of swim latencies during acquisition training for Cannabis sativa fed mice in the different experimental groups. The results in Fig. 1 show the swim latency during acquisition training. Low Dose (LD) and High Dose (HD) were significantly (p<0.05) longer on day 2 and 3 compared to control.

Comparison of swim latencies during reversal training for Cannabis sativa fed mice in different experimental groups: Results of the swim latency during reversal training showed that the LD and HD were significantly (p<0.01) and (p<0.001) longer respectively compared to control on day 2. The swim latencies were still longer for the test groups with the LD significantly longer (p<0.05) compared to control in day 3 (Fig. 2).

Comparison of quadrant duration during probe trial for Cannabis sativa fed mice in the different experimental groups: Quadrant duration refers to duration of time the animals spent in the different quadrants. In Fig. 3, there was

MATERIALS AND METHODS

Preparation of Cannabis sativa extract: The leaves of the plant were dried to evaporate its water content and blended using a manual blender into snuff-like particles and its weight taken. A solution of 80% ethanol and 20% of distilled water was prepared. The particles were then added into the solution and kept overnight (18 h). At the expiration of 18 h, the mixture was filtered using Whatman’s No. 1 filter paper into a conical flask. The filtrate obtained was dried using Astell Hearson oven at 45°C. After all the water content in the filtrate had evaporated, the extract was scrapped off and stored in an airtight container and refrigerated until used.

Animal care: Twenty seven adult CD1 mice were housed separately in metabolic cages under standard laboratory conditions in Physiology Department, University of Calabar, Calabar with room temperature of 25±2°C, where they could observe the dark/light cycle throughout the duration of the experiment. They were fed with normal rat chow and given water freely and allowed to familiarize with the environment for one week before the commencement of the experiment.

Animal treatment: Twenty seven albino mice were randomly separated into 3 groups. Group I (the control group) was given 10 mL kg⁻¹ of normal saline. Group 2, the low dose group received 10 mg kg⁻¹ day⁻¹ of Cannabis sativa while group 3, the high dose group received 20 mg kg⁻¹ day⁻¹ of Cannabis sativa. Extract was administered orally with the aid of an orogastric cannula for 28 days.

Statistical analysis: Data obtained was expressed as Mean±SEM, Analysis of data was done using one way analysis of variance (ANOVA) and Post hoc Neuman keul test with the aid of the statistical package (SPSS 15.1) and were considered significant at p<0.05.
Fig. 1: Comparison of swim latency during acquisition training for Cannabis sativa fed rats and control, values are Mean±SEM, n = 9, *p<0.05 vs. control

Fig. 2: Comparison of swim latency during reversal training for Cannabis sativa fed rats and control, values are Mean±SEM, n = 9, *p<0.05, **p<0.01, ***p<0.01 vs. control

Fig. 3: Comparison of quadrant duration during probe trial for Cannabis sativa fed rats and control, values are Mean±SEM, n = 9, *p<0.05 vs. control

significant difference in the time spent in the North East quadrant which contained the escape platform during the acquisition training. The HD and LD groups spent significantly (p<0.05) less time compared to control. There was no significant difference in the time animals in the three groups spent in the South West quadrant which had the escape platform during the reversal training.

Frequency of annulus acquisition crossing and annulus reversal crossing in the different experimental groups: The results for frequency of annulus acquisition crossing indicate that the test doses, LD and HD were significantly lower (p<0.01) and (p<0.05), respectively compared to control. There was no significant difference between the HD and LD groups. From the results of frequency of annulus reversal crossing, HD and LD had lesser frequency compared to control (Fig. 4).

Comparison of swim latencies during the visible platform task for Cannabis sativa fed mice in the different experimental groups: Results of the swim latencies during the visible platform task showed that, HD and LD test groups had longer swim latencies compared to control with HD significantly longer (p<0.05) compared to control (Fig. 5).

DISCUSSION

In order to investigate the effects Cannabis sativa (marijuana) on visuospatial learning, the Morris water maze was used. The Morris water maze (Morris, 1984) was used as a test for spatial learning in rodents. The hidden-platform version of the MWM is a test of visuo-spatial learning and memory, performance of which is impaired by hippocampal lesions (McDonald and White, 1994).
Results obtained from the hidden platform task showed that during the acquisition training and reversal training, which lasted for 3 days each the swim latencies were longer in the mice treated with low and high dose Cannabis sativa when compared to the control group. The high dose Cannabis treated mice showed significantly longer swim latencies compared to the low dose Cannabis treated mice. The longer the swim latencies, the reduced the learning and memory processes. This result suggests impaired learning ability for the two Cannabis sativa treated groups and agree with a study (Chen et al., 2000) where tetrahydrocannabinol, synthetic cannabinoids and anandamide were used on experimental animals; this animals showed deficits in short-term memory in spatial learning task and also suppression of long term potentiation in the hippocampus, a process that is essential for the formation and storage of long term memory.

During the probe trial test, there was no escape platform so that visuo-spatial memory could be assessed. Typically, a well-trained rat will swim to the target quadrant of the pool and repeatedly across the former location of the platform. This spatial bias, measured in various ways, constitutes evidence for spatial memory. The frequency of exploration in the NE quadrant showed that the mice treated with low and high dose Cannabis sativa, both had significantly lower frequencies and duration compared with control, therefore reduced learning and memory ability independent of the dose administered. No significant difference was observed in the frequency and duration of exploration in the Southwest (SW) quadrant (which had the escape platform during the reversal training) by the animal across all the experimental groups. The result in the frequency of annulus training crossing which refers to the number of times an animal crosses the quadrant that had the escape platform. The Cannabis treated groups showed a significantly lower annulus acquisition frequency compared to control, i.e. animals in the Cannabis sativa treated groups could not locate where the platform had been compared to the control group suggesting a poor learning ability, this further supports the results from the probe trial test. The visible-platform (cued) version of the MWM is a non-hippocampal task, which is disrupted by dorsal striatum lesions (McDonald and White, 1994). Here, a visible escape platform was placed in one of the quadrants of the pool to allow the animals locate it easily. This is called cue learning (Whishaw and Kolb, 1984). The Cannabis sativa treated groups showed longer swim latencies compared to control indicating an impaired visual ability. These effects observed could be as a result of the main active constituent in Cannabis sativa, THC (Pertwee, 2006). Cannabinoids also appear to work by reducing glutamate release below the level needed to activate N-methyl-d-aspartate (NMDA) receptors, a requirement for long term potentiation (Vorhees and Williams, 2006).

CONCLUSION

Long term administration of Cannabis sativa leads to impaired visuospatial learning in mice. Therefore its illegal use should be totally or completely be discouraged.

REFERENCES


