Catha edulis deteriorates spatial working memory in rats, but spares reference memory

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Abstract

The effects of Catha edulis, a CNS stimulant, on humans and animals have been studied on various aspects like anorectic effect, self-administration, stereotyped behavior, aggressive behavior, operant task, locomotor sensitization, psychosis etc., but how C.edulis influence spatial learning and memory in rats is not clear. C.edulis contains amphetamine like substances, which enhances spatial learning and memory. So, we hypothesize C.edulis will also influence spatial learning and memory. In the aim to assess this effect of C.edulis, a comparative study is conceded using another CNS stimulant, methylphenidate (MPD), which is currently used, for treatment of attention deficit hyperactive disorder (ADHD), instead of amphetamine. Spatial learning and memory was assessed using radial arm maze, by analyzing five dependent measures obtained on every trial: time to complete a trial, latency to first arm entry, number of reference memory errors, number of working memory correct and incorrect errors. Our results show that C.edulis and not MPD fed rats had impaired learning and memory, implicated by increased time to complete a trial. But both C.edulis and MPD increased attention in rats, as in both groups latency to first arm entry was less. Further analysis showed that C.edulis fed rats were more effected in the working memory component and reference memory was intact. These results highlight the importance of restricting the widespread use of C.edulis in humans. The use of MPD as a choice of drug in treatment of ADHD is also supported by this study as it did not deteriorate the learning and memory, in spite of increased attention and alertness. These results are further discussed on the basis of differential action of C.edulis and MPD on neurotransmitter systems of brain, and this reveals the need for detailed analysis in future studies for the effect of C.edulis on hippocampal network.

Introduction

Catha edulis leaves are chewed habitually in Yemen and the south-western part of the Arabian Peninsula, in the East African countries between Sudan and Madagascar, namely Djibouti, Ethiopia, Somalia, Kenya, Tanzania and Uganda. In Europe, Australia and the United States, C.edulis use is seen amongst immigrants from Yemen, Somalia and Ethiopia (1, 2). Some authors estimate that 10 million people chew C.edulis leaves worldwide (3). It is known that it has stimulatory effects due to the presence of amphetamine like substances. C.edulis contains an alkaloid called cathinone, an amphetamine-like...
stimulant, which is said to cause excitement, loss of appetite and euphoria (4). Chewing of C.edulis leaves induce a state of euphoria and elation with feelings of increased alertness and arousal (5, 6). In this study the focus is on the effects of C.edulis on spatial learning and memory in rats.

Rats fed C.edulis show increased locomotor activity and reduced weight gain due to decreased absorption of food (7). In addition, C.edulis extracts and cathinone (active content in C.edulis leaves) produce stereotyped behaviour, self-administration and anorectic effects in animal species (8, 9, 10, 11, 12). They also enhance baseline aggressive behaviour of isolated rats (13). Furthermore, cathinone is capable of producing conditioned place-preference in rats, thus showing the rewarding effect of the drug (14, 15). Cathinone is also able to act as a discriminative stimulus in a food-reinforced operant task (16). However, how the C.edulis affects the spatial learning and memory is not yet clearly analyzed.

C.edulis have amphetamine like substances, and many studies show that amphetamine influence spatial learning and memory. An fMRI investigation in humans show that processing efficiency of a verbal working memory system is modulated by amphetamine (20). In humans also, similar study showed that amphetamine enhances visuospatial working memory (21). A study in rats using one-way inhibitory avoidance learning paradigm, showed that norepinephrine plays a facilitatory role in the memory process and amphetamine enhances retention performance, at least in part, through facilitation of hippocampal norepinephrine release (22). Another study in rats using six arms RAM, and elevated T-maze showed that amphetamine exposure selectively enhances hippocampus-dependent spatial learning and attenuates amygdala-dependent cue learning (23). All these studies support the hypothesis that C.edulis, which contains amphetamine like substances, can influence spatial learning and memory, which is analyzed in this study using eight arms RAM.

In order to compare the effects of C.edulis on spatial learning and memory to another CNS stimulant, Methylphenidate (MPD), which also enhances attention and alertness similar to that of C.edulis, but possibly through unrelated mechanism of action to that of C.edulis, was used in this study. MPD is a mild CNS stimulant that blocks the transporters of the catecholamines – dopamine and noradrenaline (24). MPD is used for Attention-Deficit Hyperactivity Disorder (ADHD) as an integral part of a total treatment program. MPD is more preferred compared to amphetamine in the treatment of ADHD (24). MPDs mechanism of action appears to be similar to those of cocaine and dextroamphetamine (25, 26). In vitro, dextroamphetamine act as inhibitor of NE uptake into the nerve terminal (27, 28) and, if uptake blockade predominates it produces comparable increase in extracellular NE. Dextroamphetamine interacts with the vesicular NE transporter (29) and therefore, increases cytoplasmic transmitter to a greater extent. The mechanism behind the extremely high levels of extracellular NE observed following MPD administration has not been firmly established. MPD can facilitate various aspects of cognition including memory formation, through their actions on
noradrenergic and dopaminergic systems (30, 31). But effect of methylphenidate on spatial learning and memory is currently under deliberation and not yet considered as an enhancer of memory formation (32). But it is clear that MPD act as a CNS stimulant and increases attention. Hence in this study the second aim is to compare the learning and memory changes associated with C.edulis to that of methylphenidate, as both are considered to be CNS stimulants.

**Experimental procedures**

Wistar albino male rats 6–8 weeks old were taken from animal house of Hadhramout University College of medicine (HUCOM). The animals were maintained on reduced feed for the entire experimental duration as a motivation for food intake in the RAM. All animals were maintained in specific pathogen-free conditions according to HUCOM guidelines that follow the NIH guidelines. All animal experiment protocols were approved by the Internal Animal Ethics Committee. Experiments were designed to minimize the number of animals used and their suffering. Total of 18 rats was used in this study, with 6 rats in each group. Rats were randomly grouped into control group, C.edulis administered group, and MPD administered group.

**Drug administration to animals**

C.edulis leaves obtained from the local market were ground with water and given orally using gavage to the C.edulis administered group of rats, on all days of experiment. It was given 30 minutes prior to start of behavioural trial, at a dose of 500 mg of C.edulis leaf/kg body weight of rat. MPD administered group of rats received MPD at a dose of 3 mg/kg body weight of rat (33), given orally using gavage 30 minutes prior to RAM trial. Inspiral®-10 SR (sustained release) tablets manufactured by Ipca laboratories limited, Mumbai, were used in the present study. Each Inspiral tablet contained methylphenidate hydrochloride USP 10 mg. The tablets were powdered and mixed with sterile 0.9% w/v normal saline. Control group of rats received 3 ml of normal saline 30 minutes prior to RAM trial on all days of experiment.

**Radial Arm Maze (RAM)**

The apparatus used was a RAM (similar to that previously described by Olton and Samuelson (34)) with eight identical and equally spaced arms radiating from the central platform. The arms were 50 cm long and 10 cm wide. Each arm and central platform had separate transparent lids. The central platform diameter was 32 cm. The whole apparatus was mounted 40 cm from the ground and placed in a small, well-lit room that contained a number of visual cues that remained invariant for the testing period. Food deprivation was introduced 2 days before habituation. This entailed monitoring the food intake of the animals for 5 days prior to this time point and reducing the amount of food given to animals to 85% of their initial intake. The weights of the animals were monitored daily till the end of the habituation period. Habituation lasted for 3 days. The first day (acclimatization) animals were allowed to explore the maze for 5 min. No food pellets were given to animals in the maze. On the subsequent days (pre-training) animals were given food pellets. On the first day they were scattered on the platform, on the second day food pellets were placed in the distal end of all the eight arms. All experimental groups performed equally well during the pre-training sessions. Acquisition lasted for 20 consecutive days with one trial per day. Each trial was terminated when either all the food pellets were consumed or at the end of 5 min, whichever came first. For each animal four arms were randomly assigned to be the baited arms, with restriction that no more than one pair of adjacent arms was baited or un-baited. During each trial rats were placed in the central platform, and allowed to freely explore the maze, while the experimenter maintained the same position throughout the whole experiments. When rats enter a baited arm and collect food pellet it was noted as a correct score, but when the rats enter an un-baited arm, for the first time, it was noted as reference memory error. When the rats enter the un-baited arm again, for the second and consecutive times, it was noted as working memory incorrect error. When rats re-enter the baited arm it was noted as working memory correct error.
Data analysis

Five dependent measures were obtained on every trial: time to complete a trial (maximum of 300 s), latency to first arm entry, number of reference memory errors (defined as the number of first entries into an un-baited arm, maximum of four per trial), number of working memory correct errors (defined as re-entries into a baited arm) and number of working memory incorrect errors (defined as re-entries into un-baited arms). The distinction of working memory errors follows that of Jarrad (35).

Statistics for RAM data

The scores used for analysis were the time taken for trial completion, the latency period, the reference memory errors, the working memory errors (correct), and the working memory errors (incorrect). These five scores were subjected to separate analyses of variances with treatment (three levels: control animals, C.edulis fed animals, and methylphenidate fed animals, as the between-subject factor and days (1–20) as the within-subject factor (SPSS 17 Software). Least significant difference (LSD) post hoc test were also performed. Within subject main effects and interactions are reported together with p values. Values are means±standard deviations (S.D.) in the text and mean±standard errors (S.E.) in figures.

Results

Time taken for trial completion

The animals were first habituated for 3 days at the end of which all the animals were freely exploring the maze. During this period all groups readily consumed the food available. Time taken for trial completion decreased in all treatment groups as a function of time (main effect for Time: F (19,359) = 34.578, p<0.0001) (Fig. 1). When averaged over all the trials, the time taken was significantly different between the C.edulis and Control group (p<0.0001) and between C.edulis and MPD group (p<0.0001), but between MPD and control group there was no significant difference (p=0.063). Mean time to complete in Control (3.033±1.088 minutes) and MPD (3.275±1.082 minutes) was lower than C.edulis fed group (4.283±0.819 minutes), this shows that Control and MPD groups are faster in completing the RAM trials than C.edulis fed rats.

![Graph showing time taken to complete the trial by each group on different days of experiment.](image-url)

Fig. 1: Graph shows average time taken (in minutes) to complete the trial by each group on different days of experiment. Note that C.edulis fed groups had significantly higher time to complete the trials, indicating they are slow in completing the trials.
Latency period

Latency period for entry into first arm significantly decreased as a function of time in all treatment groups (main effect for Time; F(19,359) = 23.953, p<0.0001). When averaged across time, latency scores showed significant differences between the C.edulis and Control group (p<0.0001) and between MPD and Control group (p<0.0001), but between MPD and C.edulis group there was no significant difference (p=0.891). Mean latency period in C.edulis (2.18±0.889 seconds) and MPD (2.20±0.856 seconds) was lower than Control group (2.93±1.067 seconds), this shows that C.edulis and MPD groups are equally faster to enter the arms from the central platform of RAM, than Control rats (Fig. 2).

Reference memory errors

All groups showed reduction in reference memory error from day 1 to 20, indicated by significantly reduced reference memory errors as a function of time (main effect for Time: F (19,359) = 139.205, p<0.0001). However, reference memory errors didn’t show significant differences between the experimental groups. Mean reference memory errors were similar in Control (2.01±1.369), MPD (2.08±1.345) and C.edulis fed group (2.24±1.335) (Fig. 3). This shows that C.edulis and MPD did not influence the reference memory of rats.

Working memory errors (correct)

C.edulis fed rats (4.48±2.825) had higher number of working memory errors (correct) when compared with MPD (3.77±2.569) and Control rats (3.38±2.470) (p=0.037 and p=0.001, respectively). MPD had similar number of working memory errors compared to Control group (p=0.249) (Fig. 4). This shows that only C.edulis and not MPD has deteriorated the working memory, even though working memory errors (correct) was decreased in all groups from day 1 to 20, as shown by the significant decrease in working memory errors (correct) as a function of time [main effect for Time: F (19,359) = 220.964, p<0.0001].

![Graph](image.png)

Fig. 2: Graph shows average latency (in seconds) to enter an arm from the central platform of RAM by rats of each group on different days of experiment. Note that C.edulis fed group and methylphenidate fed group had significantly lower latency period, indicating they are fast in entering the arms.
Fig. 3: Graph shows average reference memory errors of each group on different days of experiment. Note that C.edulis fed group and methylphenidate fed group had almost same reference memory error as control group, indicating that these drugs have not influenced the reference memory.

Fig. 4: Graph shows average working memory errors (correct) of each group on different days of experiment. Note that C.edulis (Khat) fed group had significantly high working memory errors (correct), indicating that this drug have deteriorated the working memory.
Working memory (incorrect) errors significantly decreased as a function of time [main effect for Time: F (19,359) = 172.662, p<0.0001] indicating that in all groups this error decreased from day 1 to 20. When averaged across time, C.edulis fed rats (5.03±2.736) had higher number of working memory errors (incorrect) when compared with MPD (4.17±2.661) and Control rats (3.87±2.725) (p=0.015 and p=0.001, respectively). MPD had similar number of working memory errors compared to Control group (p=0.391). This again shows that only C.edulis and not MPD has deteriorated the working memory (Fig. 5).

Discussion

This study looks at the effect of C.edulis on spatial learning and memory, and by doing so an indirect indication of effect of C.edulis on brain areas involved in spatial learning and memory, especially hippocampus, is revealed. Since C.edulis contents are known to act as a presynaptic releaser and re-uptake inhibitor of dopamine (6) and cause depletion of serotonin (13, 19), it is logical to think of some effect for C.edulis in spatial learning and memory. Moreover its effect on acetylcholine metabolism is understudied and C.edulis effects of cognition is less studied than effects on psychosis. Therefore, the primary motivation behind the current study was to see if specific cognitive deficits can be associated with C.edulis administration. For this a comparative study model was used, by comparing the effects of C.edulis, which is known as a CNS stimulant causing increased alertness and arousal (5, 6), to another CNS stimulant methylphenidate, which also similarly cause increased alertness and arousal (24).

In our experimental paradigm, rats were given orally the C.edulis leaves ground in water, and not the extract or the active content of the C.edulis alone. This study design was chosen since it closely mimicked the realistic situation in population, where they consume the whole leaf and drinks a lot of water with it. Further studies using isolated...
compounds of C. edulis leaves will determine whether specific compound or combination of compounds leads to the observed results. Moreover, in this paradigm, the C. edulis and methylphenidate drug were given only during the experimental days, 30 minutes prior to the start of RAM task, this restricts the discussion to immediate effect of C. edulis and contribute less to the long term effects.

The confounding factors that could account for the deficits in the performance of the experimental groups in the RAM are motivation and motor deficits. In the current study, since food deprivation is used as a motivating factor, and since some studies shows anorectic effects of C. edulis in animal species (8, 9, 10, 11, 12), the effect of this on the different experimental groups needs to be ruled out. Our observational evidence suggests that none of the groups showed any motor deficits, and thus it is not the prime reason for the difference in performance since all groups finished their trial in less than 5 min, although there were significant differences between the groups on total trial time. Therefore, the memory errors are unlikely to be accounted for by motor deficits. Observations from our lab show that, during the food deprivation period the weights of animals in all the groups dropped such that by the beginning of the experimental period in the RAM there was no difference in the weight of the animals between the different groups. In addition, it was seen that there was no difference in the amount of food consumed in their home cages (data not shown). In addition, during the RAM acquisition period by day 15 all the animals in all the groups finished the pellets kept in the baited arms. The above results show that no consistent trend of differential food consumption or weight loss could be detected that could account for the differences in the memory scores across the different experimental groups. Therefore, the differences in cognitive performance between the groups are not likely to arise from either the differences in feeding pattern of the various groups or due to motivation issue. Thus, it can be reasonably concluded that the differences in the performances observed are indeed due to memory deficits.

Interestingly, data from Fig. 1 and 2 shows that Control and MPD groups are faster in completing the RAM trials than C. edulis fed rats. However, control group is slower to enter the arms from the central platform than the C. edulis and MPD group of rats. This clearly indicates that increased total trial time in C. edulis group of rats is not due to motivation factor or motor deficit, instead it is due to cognitive deficits, which made the rats to make more errors and thus retained in the RAM for longer duration than Control group. C. edulis leaves chewing induce a state of euphoria and elation with feelings of increased alertness and arousal (5, 6), also MPD increases alertness and arousal (24), and this may be the contributing factor for decreased latency period to enter the arms, in C. edulis and MPD groups. In spite of increased alertness, only in C. edulis group the total trial time was increased and not in MPD groups, indicating that even though both C. edulis and MPD are CNS stimulants, only in C. edulis group cognitive deficits are observed. This is probably because the mechanism of action at the neurotransmitter level is different in C. edulis and MPD. This was not investigated further in the current study.

Spatial representation is one aspect of cognition where the abilities of humans and animals have been extensively compared (36, 37). The RAM task designed in our experiments had four baited/four unbaited arms. This design enables a direct comparison between reference memory and working memory performance through a within task within subject comparison (38). In this task, memory is usually inferred from the day-to-day improvement in performances in the RAM that is indicative of reference memory of the animal. Our results show that there is clear-cut reduction in the number of reference memory errors over the training period in the control group suggesting that these rats had learned to locate and not to enter a never-baited arm. This measure is unaffected in the C. edulis and MPD fed rats. In the case of MPD, whether it enhances or deteriorates memory is still under controversy (32). But this study indicates that at least MPD does not deteriorate the reference memory. In the case of C. edulis also reference memory is unaffected, probably because the long
term potentiation happening in cortical regions, which is required for storage of memory is unaffected by C.edulis and MPD. Another possibility is that, in this study C.edulis and MPD are only given for short duration during experimental stage, and this may not be sufficient enough to cause alternation in brain circuitry that will influence reference memory. This rational for the result makes further studies obligatory to look for the long term feeding effects for both C.edulis and MPD.

Working memory is a form of short term memory that keeps information available, usually for very short periods, while the individual plans action based on it (39). In the case of RAM, an important component of working memory is the short term storage of trial-unique information, whereby unique information about specific stimuli (e.g. here in RAM spatial location of food) is retained briefly in a short-term memory buffer and discarded after an appropriate response (consumption of food, or completion of RAM trial) is executed. In RAM task used in this study, working memory is measured by counting the number of working memory errors within a particular trial. The working memory errors were further classified into working memory correct and working memory incorrect errors which reflect the animal’s ability to learn and memorize previous entries into baited or un-baited arms respectively. In this measure of memory, C.edulis fed animals was affected significantly, but MPD fed rats were similar to control rats.

Cathine (norpseudoephedrine) and cathinone [S(-)-alpha-aminopropiophenone] well account for the CNS stimulant effects of C.edulis. When C.edulis is chewed, absorption of cathinone ensue with maximal plasma concentrations occurring at approximately 2 hours (40). The terminal elimination half-life is approximately 4.3 hours. Similar effects are achieved with orally administered pure cathinone. Cathinone is the keto-analog of cathine and because it is more lipophilic it penetrates the blood-brain barrier more easily. The plasma life of cathinone is 1.5 hours (40). Since the half-life of the main CNS stimulant in C.edulis is short, this study restricts to immediate and short term effects rather than continuous and long term effect. Also the long term effects beyond the 20 days are not analyzed in this study. How C.edulis impairs working memory is not further analyzed in this study. Even though C.edulis contains amphetamine like substances, which is considered as an enhancer of learning and memory, here in this study, C.edulis instead of enhancing working memory, it deteriorates spatial working memory. Anticholinergic drugs, like scopolamine (SC), can disrupt short-term or working memory in humans and animals (41). Probably, C.edulis may also show anticholinergic activity, or may influence the hippocampal neuronal network directly. Further studies are required to confirm the cause, but it is definite that C.edulis impairs spatial working memory.

Conclusion

In conclusion, our study highlights the adverse effects of C.edulis on spatial learning and memory in rats, which may have similar effects on humans too. C.edulis specifically impairs working memory and reference memory is uninfluenced. Further, it emphasizes the difference of action of two CNS stimulants, and shows that only C.edulis and not MPD impairs spatial learning and memory. This also supports the use of MPD as an integral part of ‘attention deficit hyperactive disorder’ treatment, because MPD just enhances the attention, as indicated by the decreased latency period and doesn’t influence the learning and memory. But C.edulis, even though increases attention, it impairs working memory, so its use among humans need to be cautioned.
References

33. Ning Z, Jeremy W, Dow-Edwards DL. Oral methylphenidate...


