



EFFECTS OF CODEINE ON MEMORY AND LEARNING IN MICE

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ABSTRACT

Memory represents a biological process incorporating a variety of cognitive attributes such as the conjunction between memory of previous actions and memory of previous knowledge. Neurobehaviour is broadly used to express the idea that all experiences have both psychosocial and biological contexts. Codeine is a pain killer but also an addictive Opioid. Taken in excess, it can cause schizophrenia and organ failure. Codeine syrup addiction is a problem across Africa, with reports of addiction. This research investigates the effects of codeine on short term memory and learning using object recognition test and elevated plus maze in mice. The codeine was diluted at a concentration of 10% w/v from the stock solution 100% w/v and used for this study. Ten mice were used for this study; the mice were randomly divided into two groups of 5 mice each. Group 1 served as the control group while Group 2 mice were orally administered 0.5 ml of 10% w/v codeine for three weeks. Learning and memory tests using elevated plus maze and novel object recognition were carried out at the end of the second week of administration. The results revealed that in the elevated plus maze test, the mice administered codeine 10% w/v 0.5 ml spent more time in the plus maze compared to the control group in day 1 (57.40 ± 14.25) as compared to the control group with (19.20 ± 4.25), day 2 (87.80 ± 11.52) as compared to the control group with (12.40 ± 1.03), day 3 (54.60 ± 12.15) when compared to the control group (11.40 ± 1.03), day 4 (38.20 ± 10.37) as compared to the control group (11.80 ± 1.43). Furthermore, the novel object recognition test also revealed a statistically significant difference in discriminative index ($P < 0.05$). Codeine decreased memory and learning in mice in this study. The availability of codeine to the general public should be more regulated.

Keywords: Codeine, elevated plus maze, learning, memory, mice,

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INTRODUCTION

The human brain is a complex entity. Emotional functions and cognitive functions occur within the human brain¹. Memory represents a “biological process” incorporating a variety of cognitive attributes. Memory is the current knowledge about something that was known previously. Memory involves two forms of recall: perfect and imperfect. Perfect recall involves a conjunction between memory of previous actions and memory of previous knowledge. While imperfect recall involves some knowledge imperfection within the context of the human ability to process information [1, 2].

Codeine is an opioid and agonist of the mu opioid receptor. It acts on the central nervous system to have an analgesic effect [3, 4, 5]. It is metabolized in the liver to produce morphine which is ten times more potent against the mu receptor. Opioid receptors are G-Protein coupled receptors that positively and negatively regulate synaptic transmission through downstream signaling. Binding of codeine or morphine to the mu opioid receptor results in hyperpolarization of the neuron leading to the inhibition of release of nociceptive

neurotransmitters causing an analgesic effect and increased pain tolerance due to reduced neuronal excitability [4, 6].

Codeine is also available in conjunction with the anti-nausea medication promethazine in the form of a syrup [7]. Brand named as Phenergan with Codeine or in generic form as promethazine with codeine, it began to be mixed with soft drinks in the 1990s as a recreational drug, called 'syrup', 'lean', or 'purple drank' [7]. Codeine is used in illegal drug laboratories to make morphine [2, 7].

The data are consistent with the hypothesis that codeine may exert its action via opiate receptors and that this system participates in memory functions. Codeine metabolizes partly to morphine by the liver enzyme CYP2D6, which is subject to genetic polymorphism. It has been suggested that analgesic effects of codeine are due to the morphine metabolite [8, 9]. Codeine appeared to have some dose-dependent effect on the central nervous system that may lead to impairment as judged from a Common Technical Document (CTD), independent of measurable blood morphine concentrations. This supports the view that some codeine effects do not seem to be mediated by morphine [1].

Classically, there are three main opioid receptors, although there are other subtypes. These are all G-protein coupled and originally named mu, delta, and kappa. When opioids bind to these receptors, a series of intracellular events occur, resulting in a decreased intracellular cAMP, hyperpolarization of the cell and neuronal cells, and decreased neurotransmitter release. Within the nervous system, activation of mu receptors in the midbrain is the dominant mechanism of opioid-induced analgesia. The cough reflex is primarily mediated through the opioid receptors in the medulla [7]. The aim of this study is to investigate the effects of codeine on short term memory and learning using object recognition test and elevated plus maze in mice.

MATERIALS AND METHODS

Animals

Ten (10) mice were used for this experiment and they were randomly divided into two groups of five each as follows:

- i. Group 1 mice served as the control group and the animals were given distil water and feed only.
- ii. Group 2 mice were administered 20%w/v codeine (20 g of codeine and 80 ml of distil water), 0.5 ml/kg each for 3 weeks

The elevated plus maze test

The mice were housed with a 12-h light/dark cycle (lights on at 7:00 AM), Behavioral testing was performed between 9:00 AM and 6:00 PM. All the experimental mice were transferred to the behavior testing room 30 minutes prior to beginning the first trial to habituate to the condition of the behavior testing room. The mice were placed in the center area of the maze with its head directed toward a closed arm. The elevated plus maze test recorded using a video camera attached to a computer, which was controlled by a remote device. The number of entries into each arm and the time spent in the open arms were recorded and these measurements serve as an index of anxiety-like behavior. Mice were allowed to move freely about the maze for 10 min. The distance traveled, the number of entries into each arm, the time spent in each arm, and the percentage of entries into the open arms were calculated by the Image EP program.

Novel object recognition

Novel object recognition was used to test for short-term memory according to the method of Darke [1] and modified by Moscardo *et al.* [3]. The mice were exposed to two objects and allowed to explore these objects then one of the objects was replaced with a novel object. If memory is functioning normally, the mice would spend more time exploring the novel object than the familiar object. If exploration of all objects is the same, this is interpreted as a memory deficit.



Figure 1: Object recognition (source: the scientist magazine)

Difference index (DI): This was taken as the difference between the exploration time of the novel object and the exploration time of the familiar object.

Discriminative index (DI): This allows discrimination between novel and familiar objects but divide the difference index by the sum of exploration time of novel and familiar object.

Precognitive index (RI): this is the percentage of time spent in exploring the novel objects relative to the time spent exploring both objects.

Ethical clearance: Ethical clearance was obtained from the ethical committee on animals in Bingham University, Karu and was assigned batch number BHUAC 213

RESULTS

Statistical analysis

The results obtained were analyzed using One Way Analysis of Variance (ANOVA) of the statistical package SPSS version 26 was used and the results obtained was presented as Mean \pm Standard error of mean and $p \leq 0.05$ was taken as accepted level of significant difference from control.

It was observed that the maximum mean time was made by the mice in the group that were exposed to codeine, 10% w/v 0.5 ml on day 1, 2, 3 and 4, and the least by the control group (figure 1, 2, 3 and 4).

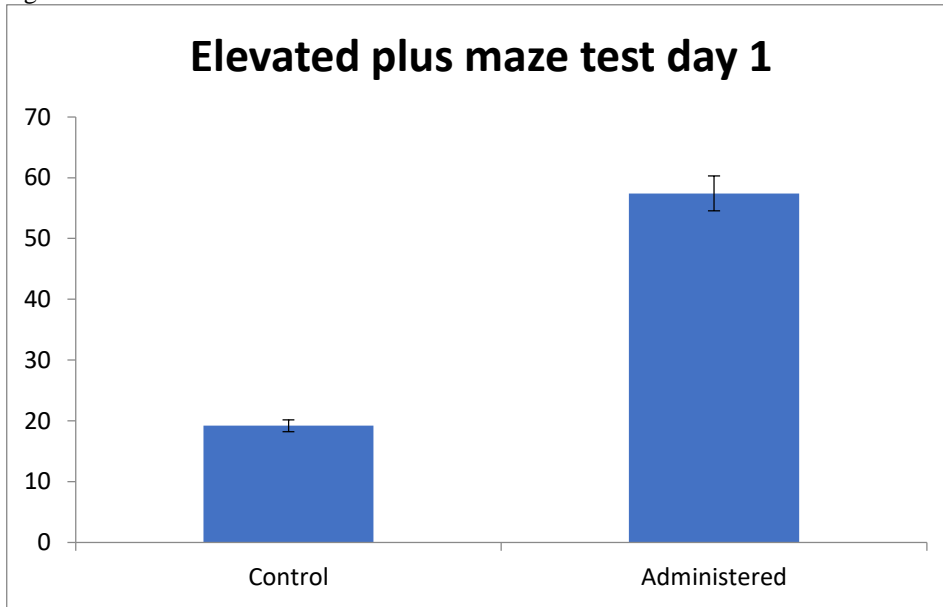


Figure 1: Effect of codeine on memory and learning in Mice using elevated plus maze test day 1.

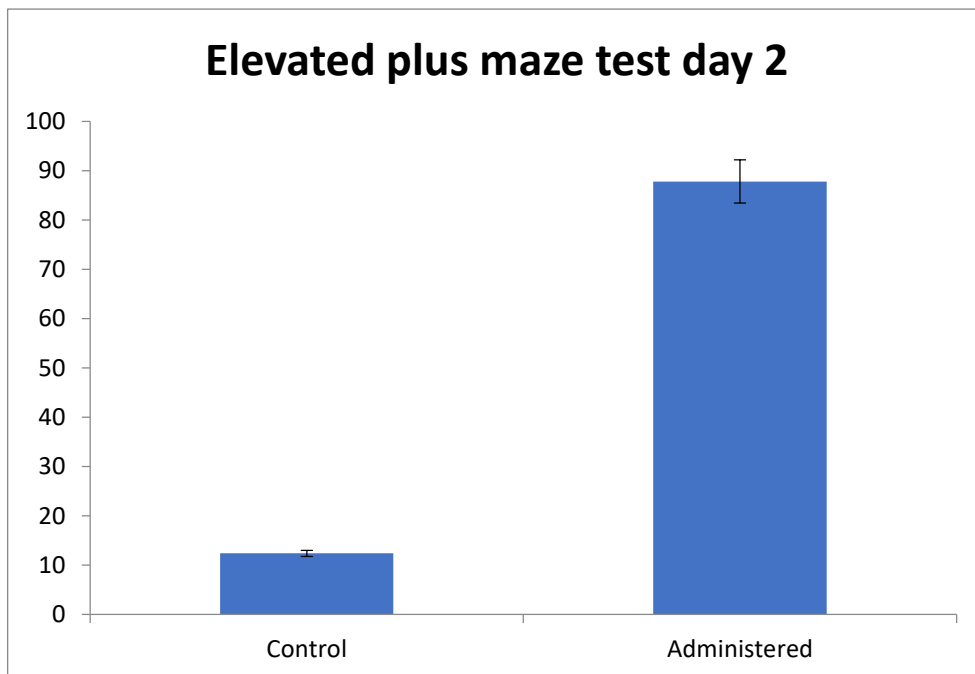


Figure 2: Effect of codeine on memory and learning in mice using elevated plus maze test day 2.

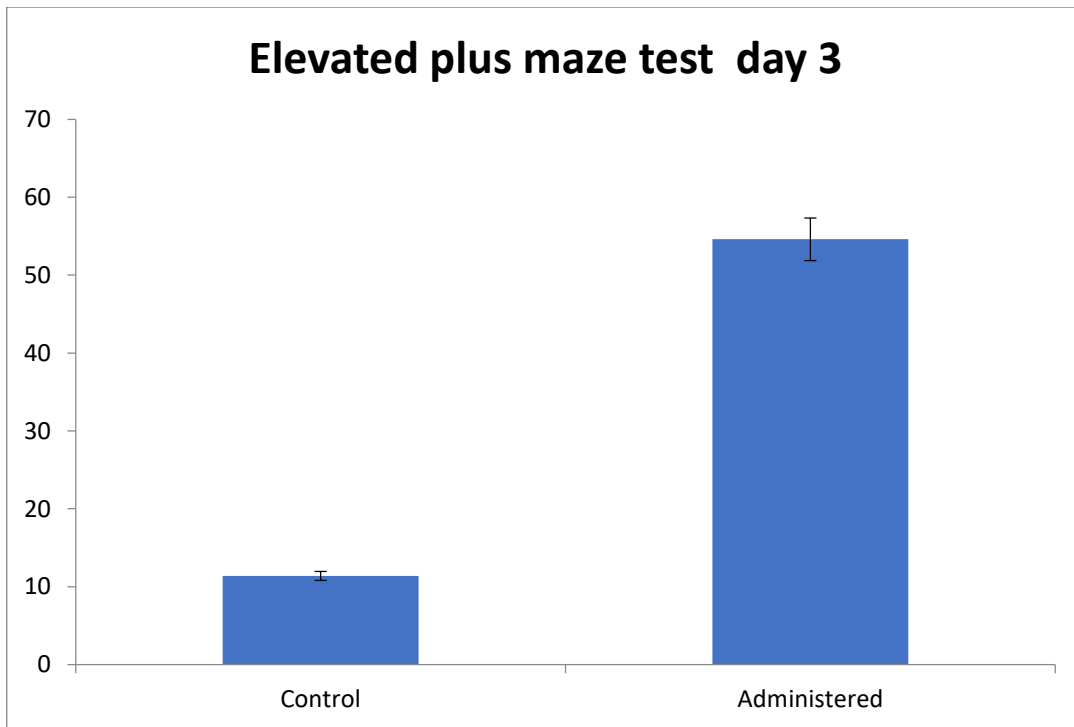


Figure 3: Effect of codeine on memory and learning in mice using elevated plus maze test day 3

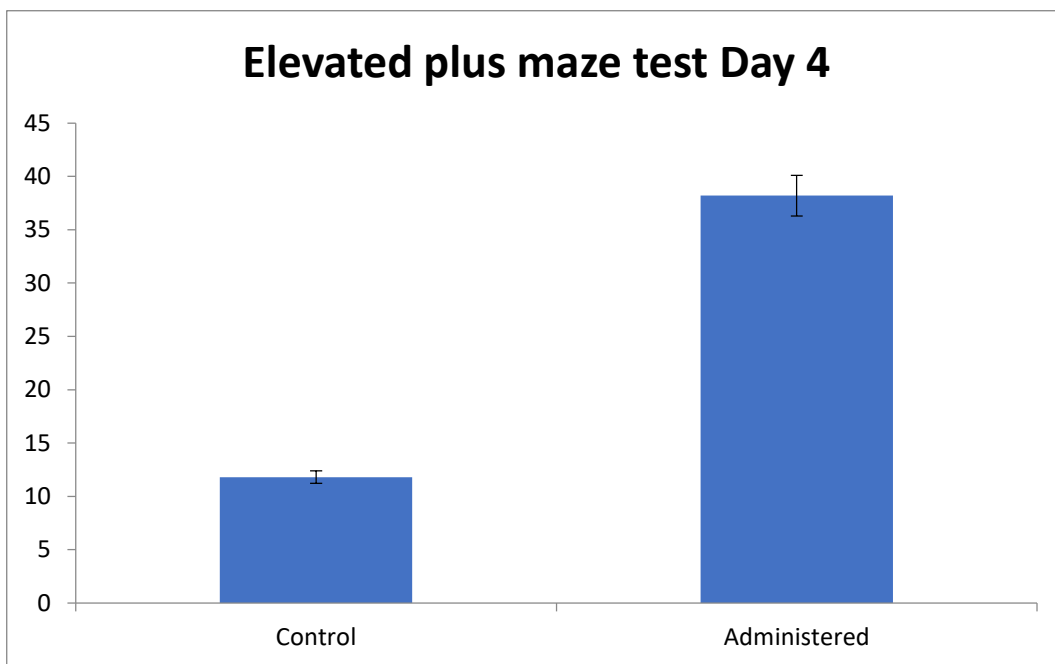


Figure 4: Effect of codeine on memory and learning in mice using elevated plus maze test day 4.

Object/Novel Recognition (Day 1)

It was observed that the positive values of Discrimination index were made by the mice in the

control group, while the least positive value was made by the mice administered codeine, 10% w/v 0.5 ml

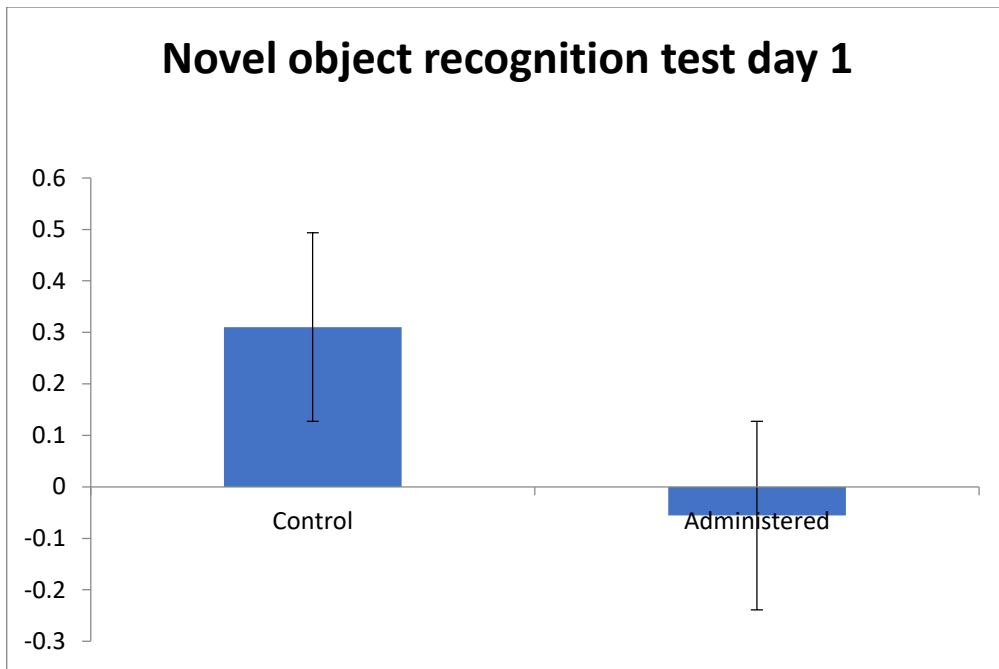


Figure 5: Effect of Codeine on novel and recognition test day 1.

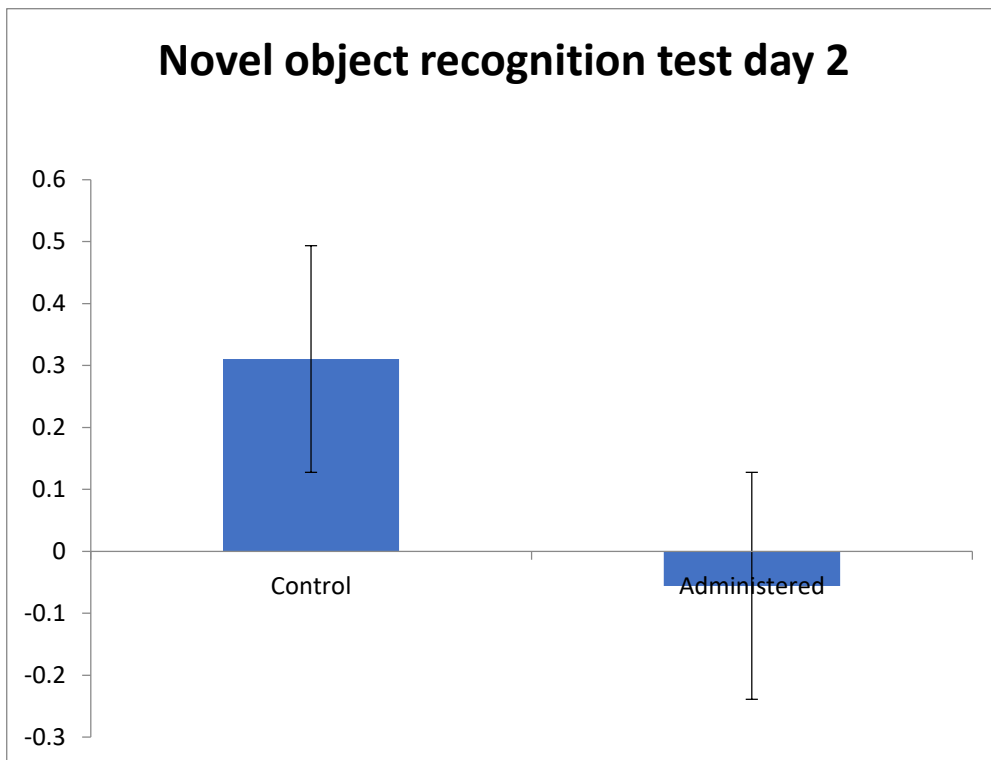


Figure 6: Effect of Codeine on Novel and recognition test day 2.

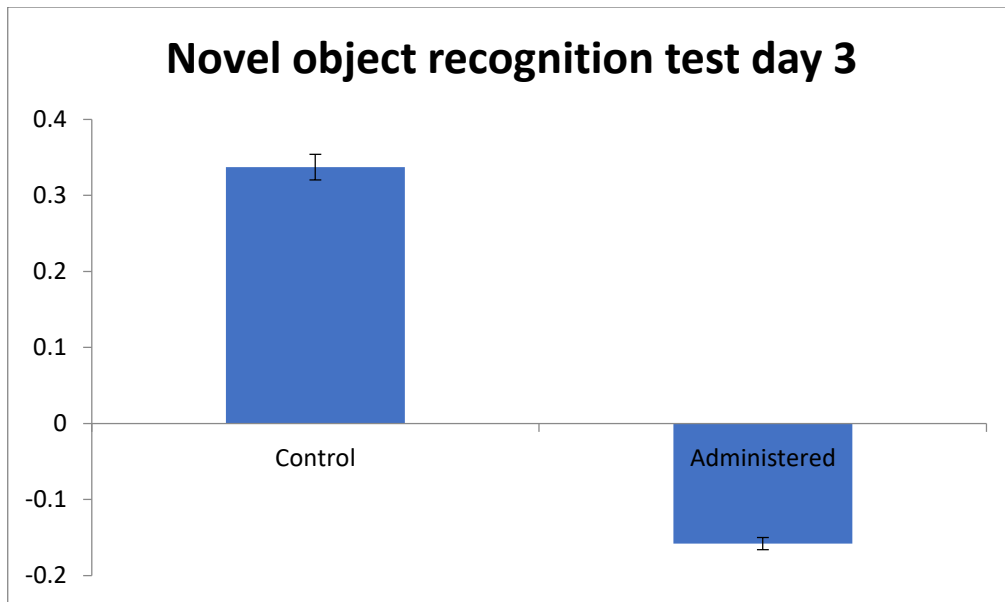


Figure 7. Effect of Codeine on novel and recognition test day 3

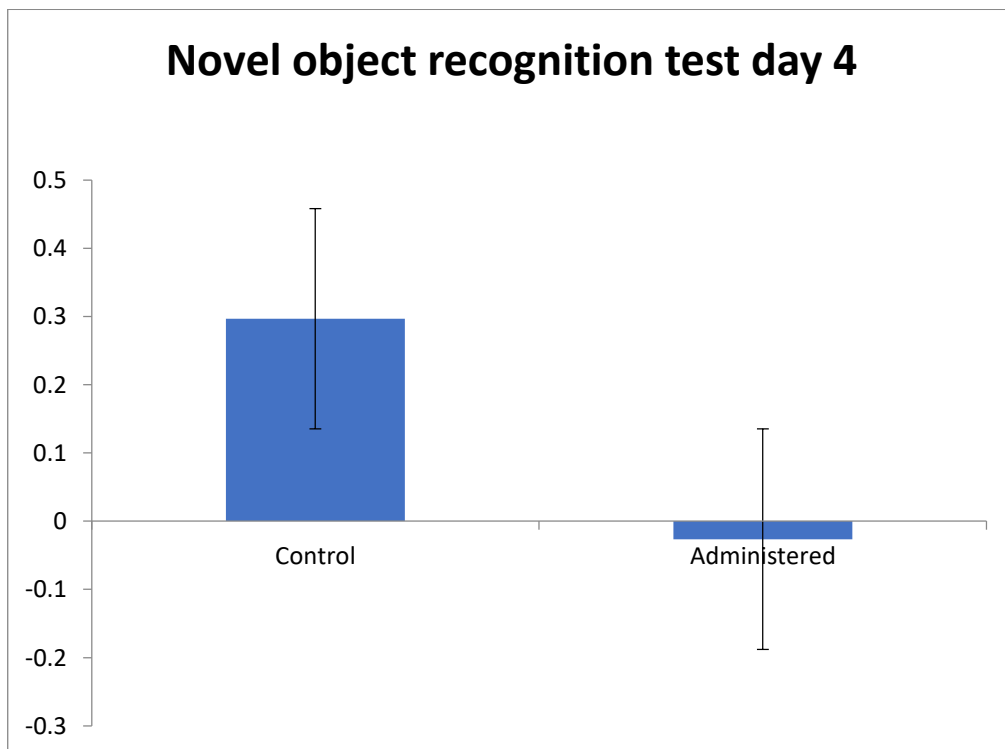


Figure 8: Effect of Codeine on novel and recognition test day 4

DISCUSSION

This study examined the effects of codeine on learning and memory in mice. The result from this study shows that mice exposed to 0.5 ml/kg single dose of codeine on the first day ($57.40s \pm 14.25s$) were slower in exploring the maze compared to the control group ($19.20s \pm 4.25s$). On the second day, there was also an increase in time spent on the maze ($87.80 \pm 11.52s$) compared to the control ($12.40 \pm 1.03s$). Furthermore, on the third day, the result

demonstrated an increase in time spent on the maze ($54.60s \pm 12.15s$) compared to the control ($11.40s \pm 1.03s$). On the fourth day of the experiment, the result shows an increase in time spent in the elevated plus maze ($38.20s \pm 10.37s$) compare to the control ($11.80s \pm 1.43s$). This result corroborates with an increase in time spent on elevated plus maze following the administration of codeine in mice reported by Kouwenberg *et al.* [6]. This can be explained basis on spatial impairment of memory after the administration of codeine [6]. Study has

shown that abuse of opiate drugs such as codeine correlates with long-lasting cognitive deficits in humans [6]. Abusers of opiates like codeine (morphine), heroin, and methadone show impairments in episodic memory, visual memory, verbal memory, information processing, problem-solving, word fluency and attention, and spatial tactile and verbal memory [8].

The Novel Object Recognition test shows significance difference ($p \leq 0.05$). In the first day, there was a decrease in discriminative index in codeine administered group ($30.60s \pm 3.44s$) compared to the control ($38.60s \pm 3.07s$) though the difference was not significant. On the second day, the result demonstrated a significant decrease in discriminative index ($20.80s \pm 11.42s$) compare to the control ($36.00s \pm 2.77s$). On the third day of the experiment, the result also demonstrated a significant decrease ($16.00s \pm 4.58s$) compared to the control ($36.20s \pm 5.74s$). Furthermore, on the fourth day the result also shows a significant decrease in discriminative index ($18.40s \pm 4.28s$) compared to the control ($31.40s \pm 4.35s$). This result concurs with decrease in discriminative index observed in novel test following codeine administration in mice reported by Osseini-Sharifabad *et al.* [9]. This can be explained on the basis that codeine administration can impair memory functions by activation of μ -opioid receptors [10]. Several studies have also indicated that chronic administration of codeine can cause histological abnormalities such as increasing apoptosis in rat cerebral cortex which is associated with oxidative stress [11].

RECOMMENDATION

Further studies should be carried out on neurochemistry to quantify the level of neurotransmitter depletion as a result of consumption of codeine. The availability of codeine to the public should be more regulated as a result of its negative effects to the mental health.

CONCLUSION

Administration of codeine impaired exploratory learning and memory in mice. These effects can be observed in the single administration of codeine. The observed effects can be justified according to the properties of codeine as opioids. It shows inhibitory effects on a wide range of different neurotransmitters and receptors like the NMDA and AMPA in addition to the stimulatory effect on the opioid, GABA, dopamine or serotonin in the brain.

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