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Effect of Caffeine at Different Concentrations on Behavior and Motor Activity in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author SMA designed the study and performed the statistical analysis. Author SSS wrote the protocol and wrote the first draft of the manuscript. Author SB managed the analyses of the study. Authors AF and NA managed the laboratory work. Author KAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This article aimed to study the effect of different caffeine concentrations on behaviour and motor activity of mice.
Place and Duration of Study: This study took place in Faculty of Pharmacy, University of Tripoli, and was conducted between 2017 to 2018.
Methodology: The experiment was carried out using 24 male mice (25-30 gm). Plus maze was used for screening antianxiety effect of caffeine. While swimming maze was used to test the antidepressant effect. Descriptive statistics was performed using SPSS (version 22), followed by one sample Kolmogorov-Simirnov test. One-Way ANOVA was applied to compare between groups and Post Hoc test (LSD).
Results: At a dose of 100 mg/kg, caffeine produce significant decrease in the duration of immobility using forced swimming maze; while the lower (25 mg/kg) and the higher (200 mg/kg) doses did not produce any changes compared to the control. In plus maze, Caffeine decreases the anxiety

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measure at the dose used of 100 mg/kg; but did not change the anxiety measure when lower (25 mg/kg) or higher (200 mg/kg) doses used compared to the control. The spontaneous motor activity was decreased significantly after administration of the higher dose of 200 mg/kg; the lower dose (25 mg/kg) showed insignificant increase, while the dose of 100 mg/kg produce insignificant decrease in the spontaneous motor activity.

Conclusion: Caffeine has dose dependent effect, in a dose 100 mg/kg it produce anxiolytic and antidepressant like action, while lower (25 mg/kg) and higher (200 mg/kg) doses did not show any changes. Caffeine also produce dose dependent decrease in the spontaneous motor activity, this indicate that caffeine produce CNS depression with higher doses.

Keywords: Caffeine; antidepressant; antianxiety; motor activity.

1. INTRODUCTION

Caffeine is classified as a stimulant drug that is typically used for its ability to arouse the central nervous system (CNS). It is generally recognized as safe by the Food and Drug Administration, at the same time, caffeine use in excess can result in health hazards and death in rare cases [1,2].

Caffeine has dose-dependent effects on mood, attention, and physiology. For example, moderate doses of caffeine (200 – 300 mg) can produce enhanced feelings of well-being, concentration improvement, and increase arousal and energy [3,4]. Higher doses (>400 mg) lead to feelings of anxiety, nausea, and nervousness [3]. Some caffeine consumers appear to develop tolerance to the negative effects of caffeine and not to the positive effects, which could lead to increase caffeine reinforcement and intake [5,6]. In humans, acute administration of moderate doses of caffeine (200 – 350 mg) decreases heart rate and increases blood pressure [7] and also increases skin conductance responses [8,9].

The behavioral effects of caffeine in humans have been well documented. Moderate doses of caffeine enhance cognitive performance [10], auditory vigilance [11] and reaction time [11,12]; these effects can be seen in doses ranging from 32 – 200 mg [11].

Our previous study (13) showed that there was excessive consumption of caffeine among local school children, and some of them suffer even severe side effects. It was found that foods and beverages consumed by children contained uncontrolled concentrations of caffeine. The levels of caffeine in the solid food and beverage samples analysed in this study were above the maximum allowable limits set by the food regulatory bodies (13). Therefore, the aim of our work is to find out the effect of higher doses of caffeine, depending on the levels found in previous study [13], on behavior and motor activity using plus maze and forced swimming maze in albino mice.

2. MATERIALS AND METHODS

The experiment was carried out using male mice (25-30 gm) bred in the animal house of the Faculty of Pharmacy, University of Tripoli. Standard mice food pellet diet and water were freely available. Mice were kept at room temperature (20-25°C) and on 12 hours dark/light cycle; animals were kept in the laboratory for at least 1 day before testing to acclimate with the new environment. Standard pure caffeine was suspended in tween 80 at different doses of 25 mg, 100 mg and 200 mg/kg.

Elevated plus-maze was used to evaluate antianxiety effect of caffeine. It was composed of two open arms (30*5 cm) and two close arms (30*5*15 cm) that extended from a common central platform (5*5 cm). The apparatus is elevated to a height of 45 cm above floor level [14]. Mice were gently handled by the right hand and placed on the center squire of the maze facing close arm. The different parameters were scored to evaluate the anxiolytic effect and spontaneous motor activity in the elevated plus-maze which include: time spent by the mouse in each of the arms, lines crossed in close or open arms and the number of entries into close or open arms. An arm entry was defined as the entry of all four paws into the arm [15]. The total line crossed, and the total number of entries was calculated. The total line crossed and the total arm entries express the spontaneous motor activity [16,17]. Anxiety measures were calculated by the time spent in the close arm divided by the total time of the test [17]. The duration of the test was 4 minutes.

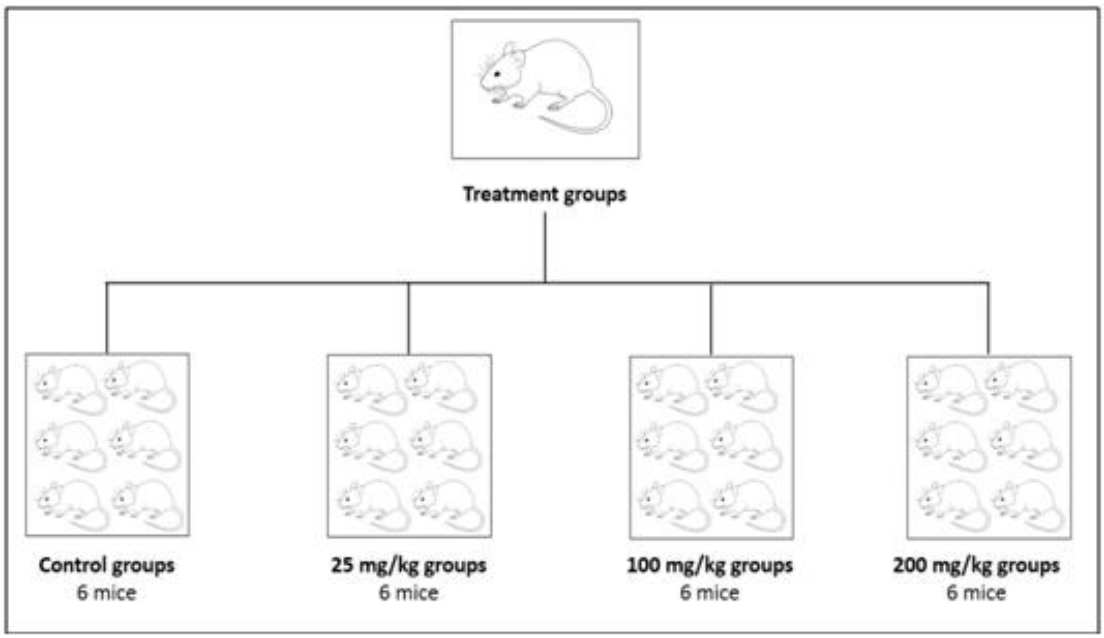

Fig. 1. Work design of the animal experiment

Forced swimming maze was used to assess antidepressant effect. In this test, mice were placed individually in glass cylinders (height 27 cm, diameter 15 cm) filled with water to a height of 16 cm (maintained at 23-25°C). The duration of the test was 6 minutes. The time spend in duration of immobility was recorded during the last 4 min of the 6 min testing period [18]. The immobility posture is characterized by floating in the water with only movements necessary to keep the nose above the surface [19].

All drugs were injected sub-acutely (three doses), mice were intraperitoneally administered at 24 hours, 5 hours and half an hour before scoring (Fig. 1).

2.1 Statistical Analysis

Descriptive statistical analyses is performed using computer program SPSS (version 22). Kolmogrove-Simirnove test maximum deviation test for goodness of fit is applied to verify whether the data were normally distributed. If the parameters are parametric, treatments are compared by one-way ANOVA and Post-Hoc test (LSD and Duncan test). If the parameters are nonparametric, treatments are compared by Mann-Whitney U test. The differences are considered significant at the P-value ≤ 0.05. The values are expressed as mean ± standard error.

3. RESULTS AND DISCUSSION

Caffeine affects many body functions, it antagonize adenosine and benzodiazepine receptors and essential enzymes like phosphodiesterase and has been shown to inhibit the release of calcium ions from intracellular stores [20].

Competitive binding of caffeine to adenosine receptors module most of the central nervous system neurotransmitters release including norepinephrine, dopamine, glutamate, acetylcholine, gamma-aminobutyric acid (GABA), and others [21].

Caffeine is nonselective competitive blockade of adenosine receptors, in particular adenosine A_1 receptors and A_{2A} receptors [22,23]. Caffeine increase dopamine (DA) signaling by blocking DA transporters and/or enhancing DA release from the terminals [24,25]; this effect is mediated by antagonizing adenosine receptors (A_1 and A_{2A} subtypes) [26,27,28]. In striatal neurons, it is known that A_{2A} R agonists decrease D_2R agonist binding [29]; caffeine, by blocking A_{2A} R, could enhance DA signaling through the unopposed D_2R [30].

Caffeine antagonism of adenosine A_1 receptors resulted in DA increases in the nucleus

accumbens [31], this finding was only obtained after very high doses of caffeine [32,33]. Volkow and his colleagues showed that there is a significant increase in D_2/D_3R availability in striatum with caffeine administration; in addition, they found that caffeine's DA-enhancing effects in the human brain are indirect and mediated by an increase in D_2/D_3R levels and/or changes in D_2/D_3R affinity [34].

In this study, the total lines crossed showed slight insignificant increase in locomotor activity; while the total number of entries was decreased, dose dependently (Figs. 2 and 3). These indicate that the locomotor activity was increased using low dose of caffeine, although it was insignificant, while caffeine with high dose produces significant decrease in locomotor activity.

Our results support previous studies. It was found that caffeine effect on motor function are highly dose dependent; it has biphasic effects, where low doses increase motor function while high doses decrease it [35-39].

It was found that the stimulant effect of low doses of caffeine is mediated by A_{2A} receptor blockade [40,41]; while the depressant effect seen at higher doses may be due to A_1 receptor blockade [41]. It was demonstrated that caffeine preferentially increases the extracellular levels of dopamine and glutamate in the shell of the nucleus accumbens (NAc) [31]. Dopamine release in either the core or the shell of the NAc, is related to the locomotor stimulant effects [42,43]. The highest dose of caffeine did not produce any effect on extracellular dopamine or glutamate levels in the shell of the NAc [31].

A study found that, the lower doses of caffeine decrease the expression of NGFI-A and NGFI-B mRNA levels in the striatum, suggesting that caffeine in low doses works as a stimulant. This effect was through blockade of adenosine receptors present in the striatum [44].

Caffeine effects depend on the administration dose. Mice treated with moderate doses of caffeine showed enhanced motor, while mice treated with high doses of caffeine showed deterioration in motor with increased anxious behavior [20]. The effects of caffeine are mediated through the antagonism of adenosine receptors, especially A_1R and $A_{2A}R$, and it exerts a stimulating effect on locomotor activity at low to moderate doses. At higher doses, however, it has even depressive effects [45,46]. While locomotor enhancement could be attributed to the multiple effects of caffeine on skeletal muscle contraction by either modulating the calcium homeostasis in the muscle fibers [47] and/or increasing the sensitivity of myofilaments to calcium ions [20,48].

It was proposed that, high concentrations of caffeine were found to interfere with the uptake and storage of calcium in the sarcoplasmic reticulum of striated muscle and to increase the translocation of Ca^{++} through the plasma membrane [47].

In basal ganglia, caffeine may affect locomotor activity; at higher doses, the balance of the two basal ganglia pathways may be disrupted, through the activation of the D_2 pathway, leading to suppression of motoric activity, or lack of coordinated movements. This is because the A_2a-D2 heteromers are the primary targets for caffeine, and inhibition of A_{2A} should lead to an increase in the actions of dopamine in the D_2 pathway, which ultimately increase inhibition of the thalamus and prevent movement [49].

Our study found that, a dose of 100 mg/kg produce anxiolytic effect; while lower and higher doses did not show any changes compared to the control (Fig. 4).

The lower dose could be sub therapeutic dose, therefore, it did not show any effect; the anxiolytic effect, produced by 100 mg/kg dose, was abolished using higher dose (200 mg/kg); this indicate that higher doses of caffeine produce anxiety, leading to abolish the anxiolytic effect induced by lower dose (100 mg/kg). Previous studies showed that caffeine cause anxiety, whereas low doses have anxiolytic effects [50-53]. Anxiolytic effects of caffeine most probably is related to agonist activity at serotonin receptors rather than antagonism of adenosine receptors [54]. While the anxiogenic behavior can be caused by blockade of benzodiazepine binding sites on $GABA_A$ receptors, stimulation of central noradrenergic activity, or antagonism of adenosine receptors [20,38,55,56].

In this study, Caffeine with the dose 100 mg/kg produce antidepressant activity; while the lower (25 mg/kg) and the higher (200 mg/kg) doses did not produce any effect (Figs. 5 and 6).

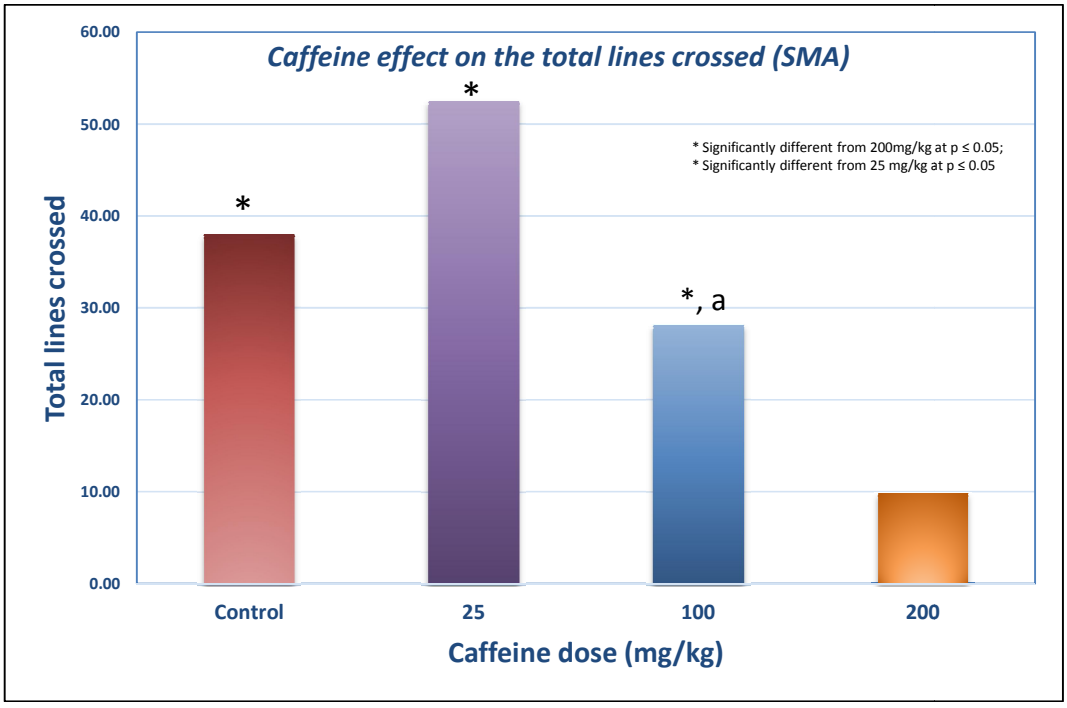

Fig. 2. Caffeine effect on the total lines crossed using plus-maze

*Test drugs: Significant from normal control, * P < 0.05; Mean ± S.E.M = Mean values ± Standard error of means of six experiments*

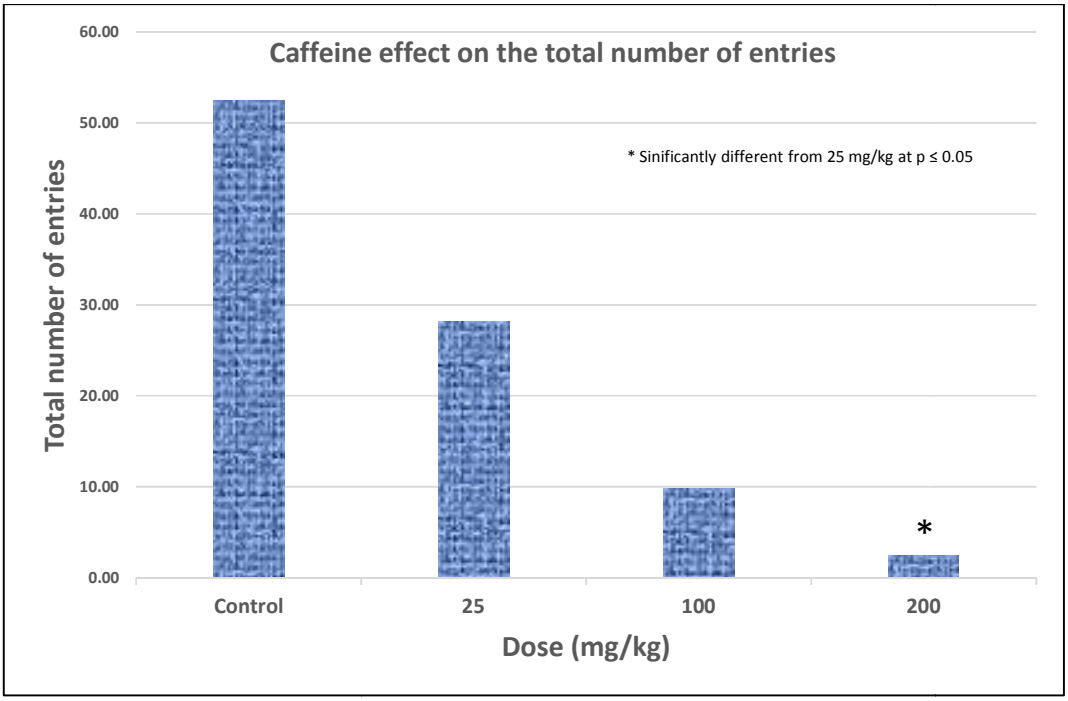

Fig. 3. Caffeine effect on the total number of entries using plus-maze

*Test drugs: Significant from normal control, * P < 0.05; Mean ± S.E.M = Mean values ± Standard error of means of six experiments*

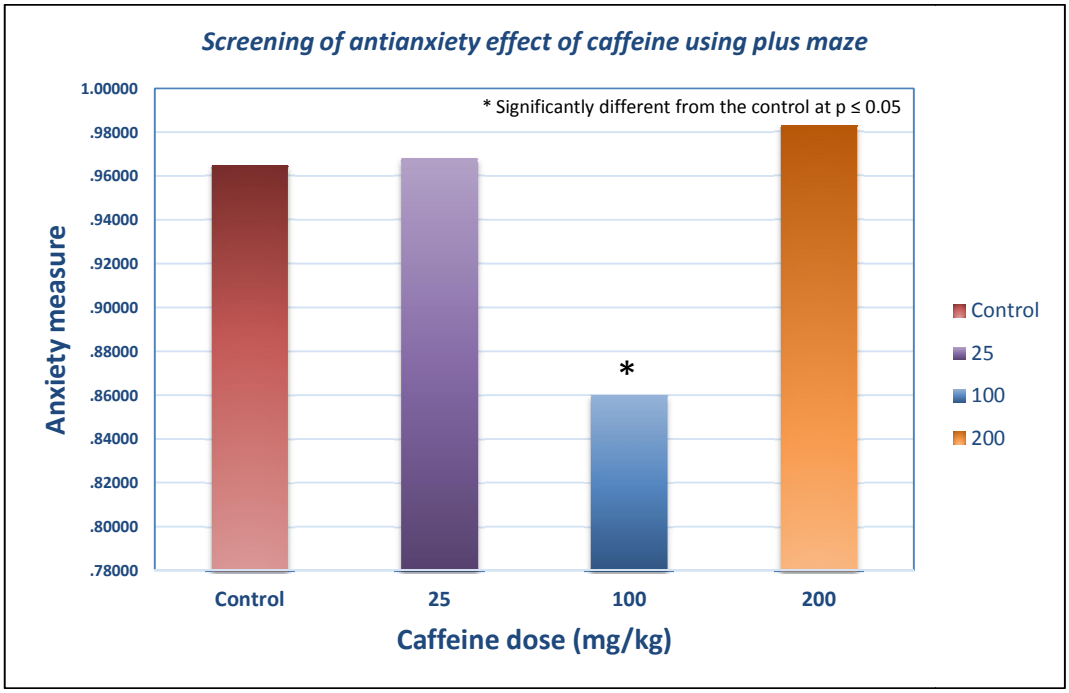

Fig. 4. Screening of antianxiety effect of caffeine using plus-maze

*Test drugs: Significant from normal control, * P < 0.05; Mean ± S.E.M = Mean values ± Standard error of means of six experiments*

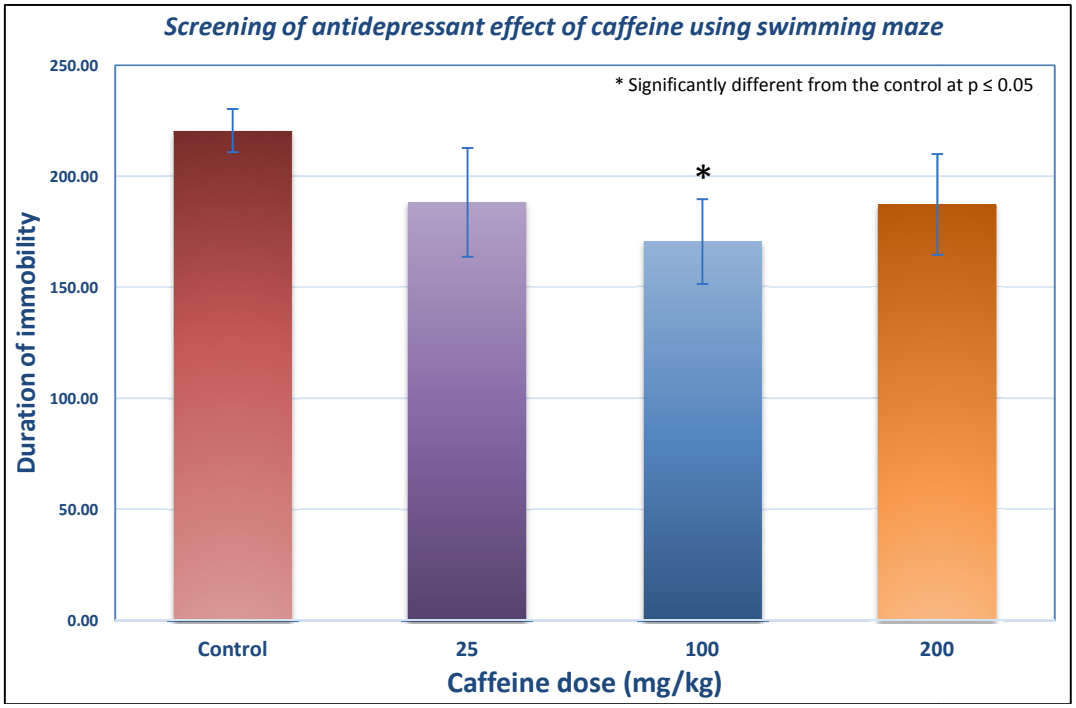

Fig. 5. Antidepressant effect of caffeine different doses against the control using swimming maze

** = significantly different from controlTest drugs: Significant from normal control, * P < 0.05; Mean ± S.E.M = Mean values ± Standard error of means of six experiments*

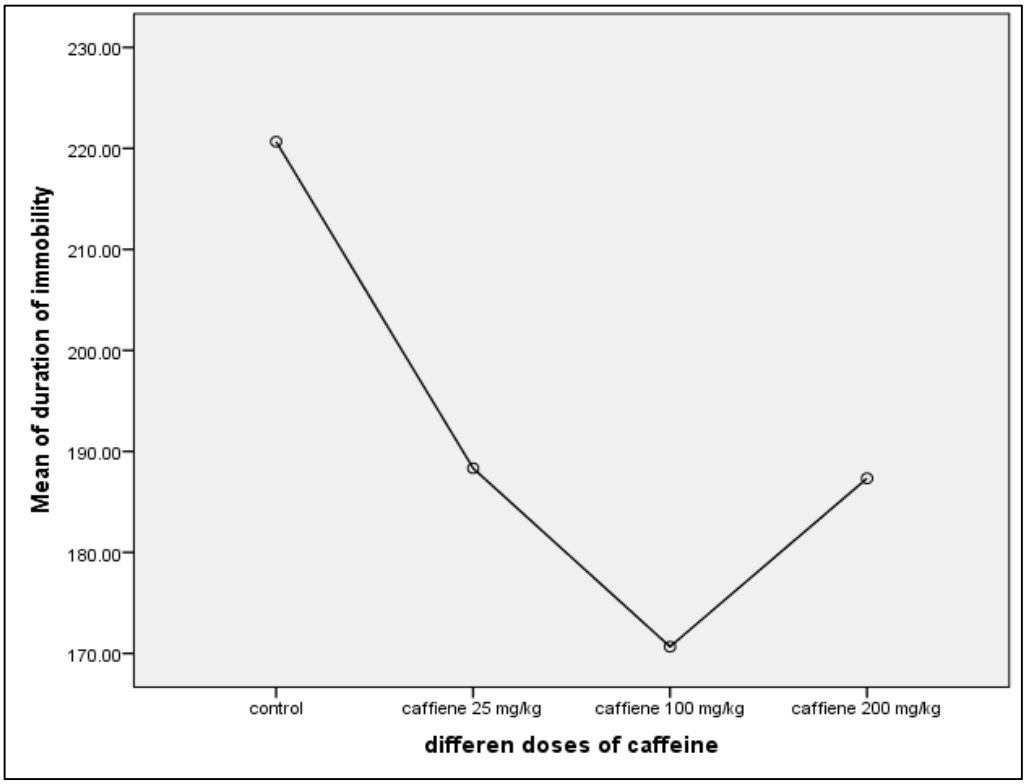

Fig. 6. Means of the duration of immobility of caffeine different doses against the control using swimming maze

Smaller dose could be sub therapeutic dose; therefore, it did not produce any significant effect. The antidepressant effect observed by caffeine (100 mg/kg) could be due to blockade of adenosine A_{2A} ARs. This antidepressant-like effect of selective A_{2A} AR antagonists, as caffeine, is probably linked to dopaminergic transmission interaction, possibly in the frontal cortex [57].

Using the higher dose (200 mg/kg) of caffeine, the antidepressant effect observed with 100 mg/kg dose was abolished. Caffeine may produce depression using higher doses through blocking A_{2A} ARs and A_1 ARs. It was found that A_{2A} ARs and A_1 ARs are involved in the antidepressant-like effect of adenosine [58] this effect, is mediated by an interaction with the opioid system, dependent on an activation of mu- and delta-opioid receptors and an inhibition of kappa-opioid receptors [59].

Another mechanism by which higher doses (200 mg/kg) may abolish the antidepressant action of caffeine of lower dose (100 mg/kg) could be explained, as caffeine is inhibitor of phosphodiesterases enzymes (PDE) leading to depression. These phosphodiesterases are responsible for hydrolysis of cyclic nucleotides cAMP and cGMP. The elevation of intracellular cAMP increases the synthesis and release of norepinephrine, which enhance central noradrenergic transmission. These effects attenuate the endogenous depression in the central nervous system (CNS) [60]. Therefore, high doses of caffeine may produce depression through the inhibition of PDE.

4. CONCLUSION

Caffiene has dose dependent effect, in a certain dose (100 mg/kg) produce anxiolytic and antidepressant like effect, while lower (25 mg/kg) and higher (200 mg/kg) doses did not show any changes. Caffiene also produce dose dependent decrease in the spontaneous motor activity, indicate that caffeine produces CNS depression with higher doses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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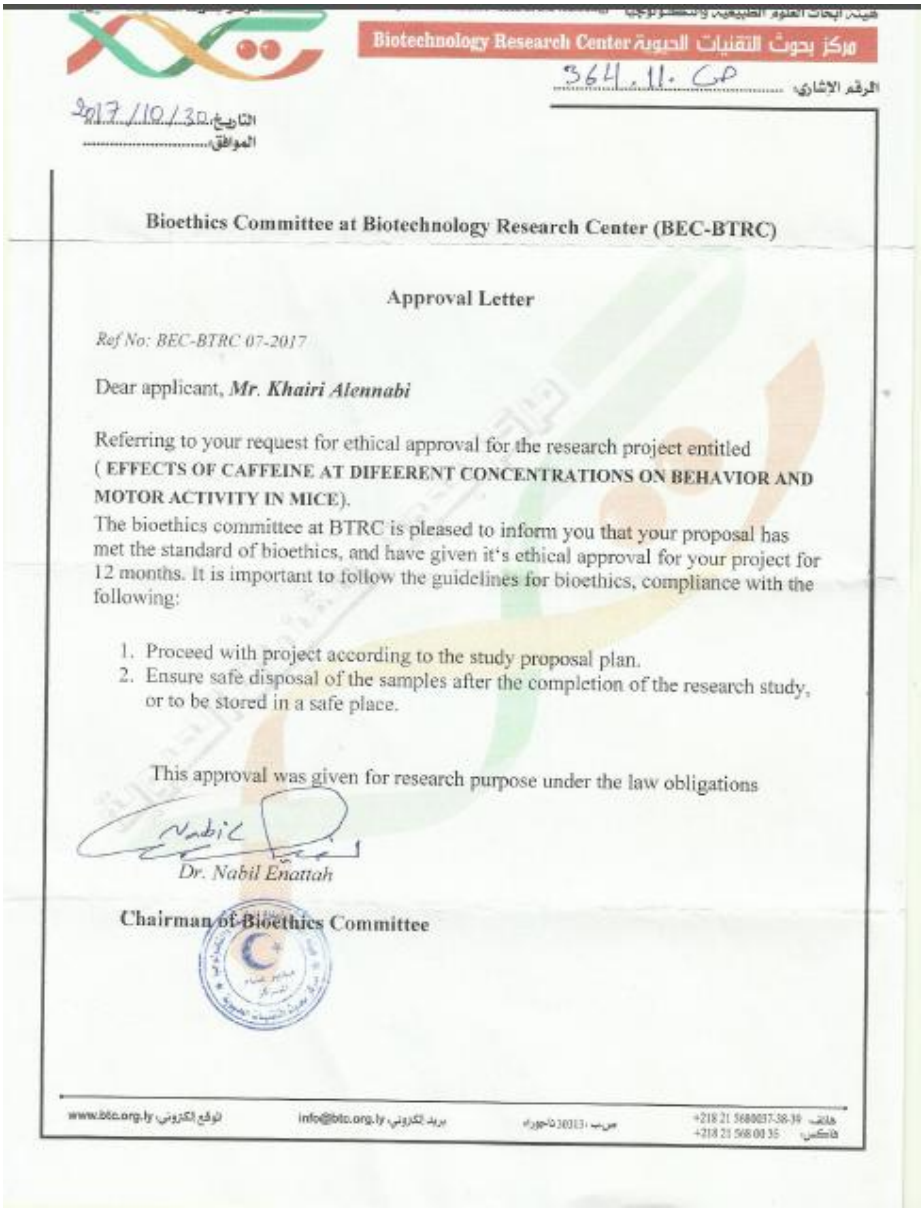
APPENDIX

Ethical approval for the animal experiment:



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التاريخ 2017/10/30
الموافق

Bioethics Committee at Biotechnology Research Center (BEC-BTRC)

Approval Letter

Ref No: BEC-BTRC 07-2017

Dear applicant, ***Mr. Khairi Alennabi***

Referring to your request for ethical approval for the research project entitled **(EFFECTS OF CAFFEINE AT DIFEERENT CONCENTRATIONS ON BEHAVIOR AND MOTOR ACTIVITY IN MICE).**
The bioethics committee at BTRC is pleased to inform you that your proposal has met the standard of bioethics, and have given it's ethical approval for your project for 12 months. It is important to follow the guidelines for bioethics, compliance with the following:

1. Proceed with project according to the study proposal plan.
2. Ensure safe disposal of the samples after the completion of the research study, or to be stored in a safe place.

This approval was given for research purpose under the law obligations

Dr. Nabil Enattah

Chairman of Bioethics Committee

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