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Evaluation of the cognitive and sleep modulating properties of paracetamol in mice

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Abstract
Cognition and sleep are important for optimum functioning of the central nervous system. Paracetamol, a commonly used analgesic and antipyretic agent is thought to modulate cognition and sleep in humans. This study was undertaken to evaluate the effect of paracetamol on cognitive and sleep indices in mice. Cognitive effect of paracetamol (250-1000 mg/kg) was evaluated using the elevated plus maze and novel object recognition tests while the diazepam and ketamine induced sleep models were used to assess its sleep modifying effects. Paracetamol significantly ($p<0.05$) decreased transfer latency in the elevated plus maze test and increased the time spent exploring the novel object. Onset and duration of sleep were increased in both the diazepam and ketamine induced test. Results suggest a modulatory role of paracetamol in cognition and sleep.

Keywords: Elevated plus maze; Diazepam; Ketamine; Novel object recognition test; Paracetamol

INTRODUCTION

Cognitive impairment or deficit is an inclusive term, which describes any feature that acts as a barrier to the cognition and memory processes. Cognitive impairment could be a deficit in global intellectual performance, intellectual disabilities or drug induced cognitive and memory impairment [1,2].

Sleep is a human act, which is important for the maintenance of normal physiological processes in the body. Sleep helps to maintain mood, memory and cognitive processes, and plays a pivotal role in normal functioning of the endocrine and immune systems [3].

Acetaminophen commonly known as Paracetamol (PCM), a commonly used analgesic and antipyretic agent is a household name in many homes [4]. Emerging studies indicate a role for PCM in memory recall and formation; and in animal studies, improved cognitive performance, anxiolytic and antidepressant activity involving cannabinoid receptors were reported in preclinical and clinical studies [5-8]. While some studies report improved cognition with therapeutic doses of PCM others report cognitive deficits at sub-therapeutic doses [9]. PCM is thought to be to be a pro-drug with analgesic PCM metabolites and a central mechanism of action involving several neurotransmitters and serotonergic, opioidergic, vanilloid, and

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cannabinoid receptor pathways. The link between analgesic, antipyretic and improved cognitive performance is yet to be elucidated [9-16]. There are conflicting reports on the effects of PCM on sleep; while PCM is thought to potentiate sleep indirectly via its analgesic property, others attribute no sedative properties to PCM [17-19].

This study was therefore carried out to evaluate the effects of PCM on (i) memory and cognition (ii) onset and duration of sleep in murine models of these indices.

EXPERIMENTAL

Drugs and chemicals. Carboxymethyl cellulose obtained from BDH Ltd Poole, England; paracetamol powder from May and Baker; distilled water; diazepam from Swipha Pharmaceuticals, Lagos, Nigeria; ketamine and piracetam purchased from Sigma Aldrich, USA were used for this study.

Animals. Swiss albino mice of both sexes (16.5-25 grams) procured from the Animal House Facility of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria were used for the study. They were kept in polypropylene cages at the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained under standard laboratory conditions (optimum temperature and humidity) and were fed with standard laboratory animal feed and clean water *ad libitum*. Handling of the animals was done according to standard protocols for the use of laboratory animals of the National institute of Health [20]. Institutional approval was obtained from the Ethics Committee of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Behavioural studies.

Elevated plus maze. The method described by Komada *et al.* [21] was used in this study. Albino mice were randomly distributed into five groups of five mice per group. Mice in group I received oral piracetam (20 mg/kg); those in group II mice were administered oral carboxymethyl cellulose (0.5%) while groups III, IV and V mice were administered oral paracetamol 250, 500 and 1000 mg/kg respectively. An hour after administration, each mice was placed in one of the open arms of the maze with its back to the centre of the maze. The latency time (time taken for a mouse to enter a closed arm with both limbs) was recorded by trained observers blinded to treatment. Mice, which did not enter any of the closed arms, were guided into a closed arm using a non-invasive object. The same procedure was repeated after an hour; recording the latency periods. The apparatus was wiped with 70% alcohol after each animal was removed.

Novel object recognition task model. Following the method described by Ennaceur and Delacour [22] with some modifications, albino mice were divided into 5 groups of 5 mice each. Mice in group I were administered oral piracetam (20 mg/kg), group II mice were administered oral carboxymethyl cellulose (0.5%) while mice in groups III - V mice were administered paracetamol 250, 500 and 1000 mg/kg respectively orally. One hour post drug administration, each mouse was placed in the open field and allowed to explore the for a minute. Mice were again placed in the field with two identical objects and allowed to explore the objects for 5 minutes. Thereafter, one of the objects was replaced with a dissimilar object and mice were placed again in the open field. Time spent exploring, licking or pawing novel object was recorded by trained observers unaware of treatment. The apparatus was wiped with 70% alcohol after each animal was removed.

Diazepam-induced sleep in mice. The method described by Beretz *et al.* [23] and modified by Rakotonirina *et al.* [24] was adopted in this study. Mice were randomly divided into four groups each of 6 mice per group. The first group received normal saline (10 ml/kg). The second, third and fourth groups were given paracetamol suspension at doses of 250, 500 and 1000 mg/kg. Thirty minutes post-treatment, the mice were administered diazepam at a dose of 20 mg/kg. The mice were placed individually in separate cages. The onset and the duration of sleep were determined for each animal. The time interval between diazepam administration and loss of righting reflex was considered as the criterion for onset of sleep [25] while the interval between the loss and the recovery of righting reflex was regarded as the duration of sleep [26].

Ketamine-induced sleep model in mice. Thirty mice were randomly divided into five groups of six mice each. The first group received normal saline (10 ml/kg). The second, third and fourth groups were given paracetamol suspension at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg. The fifth group received diazepam at 0.5 mg/kg. Thirty (30) minutes post-treatment, the mice were administered ketamine at a dose of 100 mg/kg [27]. The mice were placed individually in separate cages and the onset and the duration of sleep were determined for each animal. The time interval between diazepam administration and loss of righting reflex was considered as the criterion for induction sleep [25] while the interval between the loss and the recovery of righting reflex was regarded as the duration of sleep [26].

Statistical analysis. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test using Graphpad Instat $^{(R)}$ Version 6. A difference was considered significant at $p < 0.05$.The results are presented as mean ± standard error of mean (SEM).

RESULTS

Elevated plus maze. The results of latency transfer time are shown in Table 1. Higher doses (500 and 1000 mg/kg) of PCM decreased mean transfer latency, this was significantly different ($p<0.05$) from the vehicle treated animals.

Novel object recognition test. In the novel object recognition test, 500 mg/kg dose level gave the highest mean exploration time followed by 250 mg/kg, these were significantly different ($p<0.05$) from the control group. Data is presented in Table 2.

Diazepam-induced sleep in mice. Paracetamol (250 and 1000 mg/kg) significantly increased onset of sleep while increased duration of sleep was observed at 500 and 1000 mg/kg dose levels. Data is shown in Fig 1.

Ketamine-induced sleep in mice. Paracetamol significantly increased onset of sleep at all doses while highly doses (500 and 1000 mg/kg) significantly increased duration of sleep. Data is presented in Fig 2.

Table 1: Effect of paracetamol in the elevated plus-maze

Treatment	Dose (mg/kg)	Mean Latency Transfer Time (s)
Carboxymethyl cellulose	0.5%	20.20 ± 3.93
Piracetam	10	14.40 ± 3.78
Paracetamol	250	41.40 ± 5.51
Paracetamol	500	14.40 ± 3.32*
Paracetamol	1000	12.20 ± 2.09*

*Values are represented as Mean ± SEM; n = 5; *p<0.05. One way ANOVA and Dunnett's post test*

Table 2: Effect of Paracetamol in Novel Object Recognition Test

Treatment	Dose (mg/kg)	Mean Exploration Time (s)
Carboxymethyl cellulose	0.5%	8.00 ± 2.16
Piracetam	10	28.00 ± 3.85
Paracetamol	250	12.00 ± 2.00*
Paracetamol	500	21.00 ± 2.38*
Paracetamol	1000	6.00 ± 0.00

*Values are represented as Mean ± SEM; n = 5; *p<0.05. One way ANOVA and Dunnett's post test*

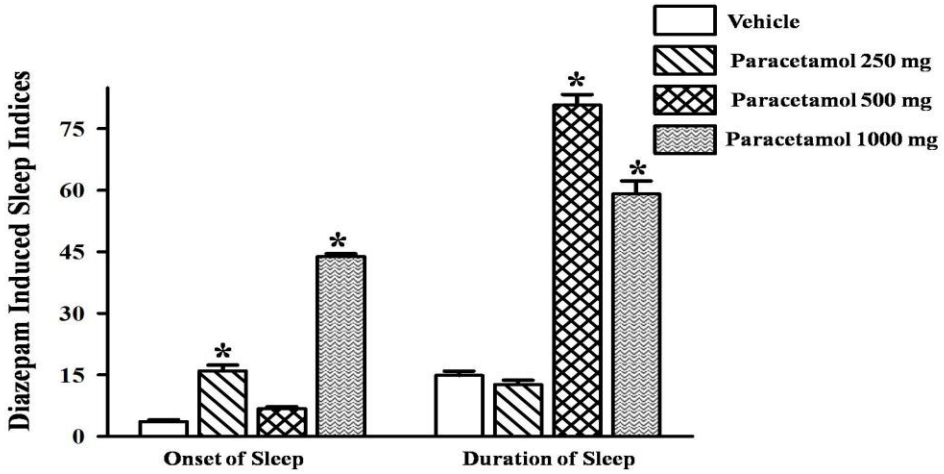

Fig 1: Statistical analysis of onset and duration of sleep in the diazepam induced sleep test. Paracetamol at 250 and 1000 mg/kg increased onset of sleep while 500 and 1000 mg/kg dose levels increased duration of sleep. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=6 per group

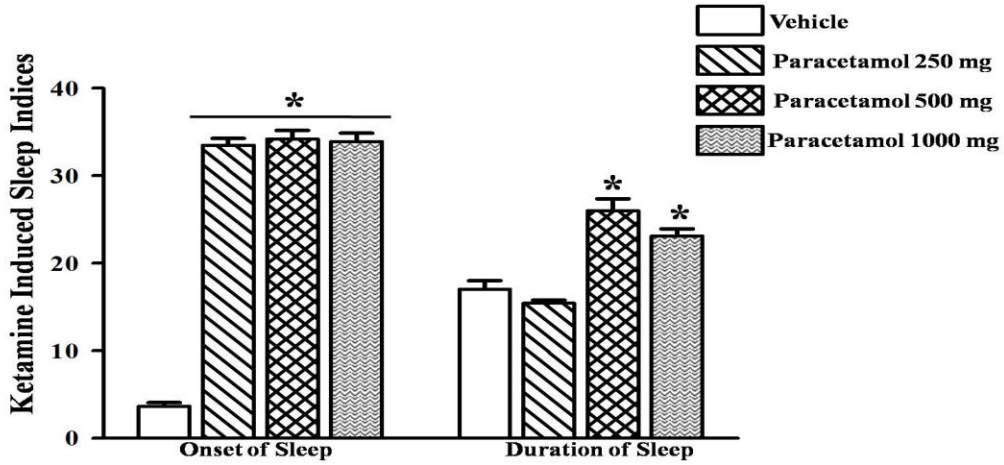

Fig 2: Statistical analysis of onset and duration of sleep in the ketamine induced sleep test. Paracetamol at the three dose levels increased onset of sleep while 500 and 1000 mg/kg dose levels increased duration of sleep. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=6 per group

DISCUSSION

Paracetamol, a commonly used analgesic was investigated for cognitive and sleep modifying effects. The elevated plus the elevated plus maze, a validated test for evaluation of memory and cognition in laboratory animals is based on rodents' natural aversion for open and high spaces. In this test, changes in latency time from the open to closed arm are indicative of memory and learning; a decrease in transfer time to the closed is taken as an index of good cognitive function while an increase implies cognitive dysfunction [28,29]. In this study, higher doses (500 and 1000

mg/kg) decreased latency transfer time into the closed arm, indicative of enhanced memory.

The novel object recognition task is a widely used model for the investigation into memory alterations and can be configured to measure working memory, attention, anxiety, and preference for novelty in rodents [30,31]. It is based on the fact that when animals are exposed to a familiar and a novel object, they frequently approach and spend more time exploring the novel than the familiar one [32,33]. The novel object recognition task is particularly attractive because it requires no external motivation, reward, or punishment and it can be completed in a relatively short time, though a little training or habituation is required [30]. In the novel object recognition test, 250 and 500 mg/kg dose levels increased time spent exploring the novel object. These findings are in line with those of Ishida *et al.* [5] and Pickering *et al.* [9] who demonstrated improved cognitive function in preclinical and clinical studies.

Diazepam acts by potentiating gamma amino butyric acid (GABA) - the major inhibitory neurotransmitter in the brain known to favour sleep - via a modulatory binding site of GABA-A receptors [34]. Glutamate receptor antagonists such as ketamine and riluzole has been documented to potentiate sleep in preclinical studies [35,36]. The lowest and highest dose of PCM used in this study, increased onset of diazepam-induced sleep while the middle and highest dose significantly increased duration of sleep. In the ketamine induced sleep test, all three doses of PCM increased onset of sleep while higher doses increased sleep duration. Increase in onset of diazepam and ketamine induced sleep suggest that PCM may not posses sleep inducing properties while potentiation of diazepam and ketamine induced sleeping time is indicative of sleep maintaining properties of PCM [24]. More studies on the effects of PCM on sleep onset and duration and elucidation of possible mechanisms of action will be undertaken.

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