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Research Article

Assessment of Cognitive and Motor Endurance Activities in Male Wistar Rats Administered Carboxymethyl Cellulose

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ABSTRACT

Carboxymethyl cellulose (CMC) is generally believed to be biologically inert, non-toxic and non-allergic. Due to its bio-compatibility, bio-degradability and other rheological properties, it has found various uses in different aspects of human life, bio-medically and industrially; that includes, suspension of physiological extract (as a vehicle), drug delivery system, tissue regeneration, formation of smart materials, hydrogels, bone growth, surgical, ultrasound procedures and foodstuff formulations (thickening, binding), lubricant for drilling in oil industry and as a stabilizer and binder in cosmetic industry. In this study, we investigated the physiological effect of CMC on cognition and motor endurance. Wistar rats were orally administered with CMC, at 5mg/kg, 10mg/kg and 20mg/kg doses while a control group was given normal saline. Cognitive function was evaluated using novel object recognition task while motor endurance function was assessed using forepaw grip test. The results obtained showed that there was no significant difference ($p > 0.05$) in both working and long-term memory in all the groups when compared to the control. Although, not significant the group receiving 5mg/ml CMC showed a lower preference score (working memory) when compared to other groups. However, at the doses 5 mg/kg and 10 mg/kg, motor endurance was significantly improved. Findings in this study suggest that although CMC may not influence cognition in this study, it may not be completely physiologically inert since it influenced motor endurance which may not be undesirable, more studies should be carried out to ascertain its neurobehavioural activity, while caution is encouraged in its usage.

Keywords: *Carboxymethyl cellulose, learning, memory, motor endurance, inert*

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INTRODUCTION

Cellulose is the most abundant regenerated biopolymer on earth, with annual production of about 5×10^{11} metric tons (Qiu *et al*. 2013; El-Sakhawy *et al*. 2014). It is an abundant naturally occurring polymer of glucose, found in plants and natural fibres such as cotton and linen (Reeves *et. al*. 2010). Some bacteria (e.g. Acetobacterxylinum) are also able to synthesize cellulose (Hokannen *et al*. 2015). Bacterial cellulose is chemically identical to plant cellulose, in both bacterial cellulose and plant cellulose, the glucose units are held together by 1,4-β-glucosidic linkages, which account for the high crystallite of cellulose and its insolubility in water and other common solvents (Lee *et al*. 2009; Sannino *et al*. 2009). Hydroxyl groups of cellulose can be reacted to form esters or ethers of different physical and chemical properties suitable for various applications. Cellulose derivatives have significant roles in industry; they represent a main source for fibers, textiles, coatings, thermoplastic films, food additives and

pharmaceutical technologies alongside with medical and biomedical applications (Reeves *et al*. 2010).

Carboxymethylcellulose (CMC) is a water soluble derivative of cellulose, it is a viscous medium, cheap, non-toxic and non-allergic typical hydrocolloid (Ke *et al*. 2014). It is obtained via etherification of cellulose involving the reaction of the hydroxyl groups of cellulose with organic species, such as methyl. Because of its rheological properties and its low cost CMC is wide application in food industry, product development and other industrial processing (Mohod *et al*. 2011;Yadollahi *et al*. 2015). It is generally used as sodium salt; Sodium carboxymethyl cellulose (NaCMC) (Sandhu *et al*. 2012; Aliu *et al*. 2016). It is used as a medium for the formation of physiological solution and suspension of drug (vehicle), serving as a medium for suspending extract. Besides carrying the drug or giving it a bulk, it can also substantially influence it bioavailability (Heinze, 2005; Mohod *et al*. 2011; Sandhu *et al*. 2012; Caramella *et al*. 2015; Mansuri *et al*. 2016).

Medically, CMC is used in ear nose and throat (ENT) surgery and used as a sodium hyaluronate and carboxymethylcellulose bioresorbable membrane in abdominal surgery to prevent intra peritoneal adhesion (Numanoglu, 2005; Otake *et al*. 2008; Sannino *et al*. 2009; Greenawalt *et al*. 2011). Carboxymethylcellulose is industrially used in food processing (as an additive), gelling (like in tooth paste), thickening and binding, in oil industry used as a lubricant for drilling and as a stabilizer, and as emulsion stabilizer in cosmetic industries (Aliu *et al*. 2016; Hokkanen *et al*. 2016).

Although there are many reports on various application of CMC and its significance in medical and industrial applications. There are few studies about the effect of CMC on the physiological systems (Brick, 1952; Anderson *et al*. 1986), there are fewer studies on its effect on the nervous system including brain and other control systems. Certain undesirable effects were observed in a previous study where Carboxymethyl cellulose was administered as a control group which were not clearly understood (Muhammad *et al*. 2014). This study was designed to investigate the possible effects of CMC on cognition and motor endurance.

MATERIALS AND METHODS

Drug: The following drugs were used in this study; Carboxymethyl cellulose (Product No: 27929, BDH laboratory, BDH Chemical Limited Poole, England) which was generously donated by the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

Animals: Male Wistar rats with a average weight of 180g, were used in this study. The animals were obtained from the Animal house, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The rats were housed, five animals in each standard laboratory cage. During the experiment the rats had access to food and water ad libitum. The animals were allowed to acclimatize to the environment for two weeks prior to the commencement of the experiment. All experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No.80-23, Revised 1996) and the Ahmadu Bello University research policy, ethic and regulations governing the care and use of experimental animals (2010).

Experimental design: The animals were randomly divided into four (4) groups each comprising five (5) animals; rats in Group I received normal saline only. Rats in Group II, III and IV received 5 mg/kg,10 mg/kg, 20 mg/kg per body weight, carboxymethylcellulose orally for a duration of 14 days.

Neurobehavioural Assessments: The neurobehavioural assessments were done after two weeks of oral administration of CMC.

Novel object recognition test: The cognition of the animals was evaluated using novel object recognition test (NOR). NOR is based on the innate tendency of animals to preferentially explore novel objects over familiar ones (Ruby *et al*. 2013; Grayson *et al*. 2015). The apparatus consisted of a rectangular open box made of plastic with a floor (31 cm × 24 cm) and 45.5 cm walls. Basically, there are two trials. In the first trial (T1) each animal was exposed to two identical objects (sample object). Following the sample object exposure, the animals were returned to their home cage for a retention period. In the second trial (T2), which follows the retention time, each animal was returned to the environment and presented with a familiar (sample) and a novel object. When the animal 'remembers' the previous exposure to the familiar object, it will explore the novel object to a greater degree than that of the familiar one.

Experiments were performed in a dimly lit and quiet room with observers inside the room. All experiments were performed between 9:00 and 14:00h. The apparatus was cleaned with methylated spirit solution between subjects.

Novel object recognition for working memory: Animals were allowed to freely explore the arena and objects for 5 min and then returned to their home cages. After an hour interval from the test (sample phase), one of the familiar objects from the sample is replaced with a novel object in the arena. Animals were allowed to explore for another 5 minutes during this test phase. Between each trial, the objects were removed, and both objects and the arena were cleaned with 70% ethanol, dried, and ventilated. For the test phase, placement of the novel object was alternated from the left to right corner from trial to trial to prevent spatial biases in object exploration.

Novel object recognition for long term memory: Animals were allowed to freely explore the arena and objects for 5 min and then returned to their home cages. After a 24 hours interval, one of the familiar objects from the sample phase was replaced with a novel object in the arena. Animals were allowed to explore for another 5 min during this test phase. Between each trial, the objects were removed, and both objects and the arena were cleaned with 70% ethanol, dried, and ventilated. For the test phase, placement of the novel object was alternated from the left to right corner from trial to trial to prevent spatial biases in object exploration. Preference score was calculated as follows:

$$\text{Preference score} = \frac{TN}{TN+TF} \times 100$$

Where; TN= time taken exploring novel object and TF= time taken exploring familiar object

Forepaw grip: The forepaw grip time as described by Abou-Donia *et al*. (2001) was used to evaluate the effect of Carboxymethylcellulose on motor endurance of the rats. This was conducted by having rats hang down from a 5 mm diameter wooden dowel gripped with both forepaws. A soft platform was placed below to prevent harming the rats. The time (Latency in seconds) spent by each rat before releasing their grips was recorded as an index of motor endurance.

Statistical Analysis

Data obtained in the study were expressed as mean ± SEM. Statistical analysis was done using SPSS version 17 and all the analysis was done using one way ANOVA followed by Tukey's post hoc test for multiple comparisons. Values of $P < 0.05$ were considered significant

RESULTS

Working memory

The results obtained for working memory is represented in figure 1. Preference score (working memory) for CMC 5 mg/kg, 10 mg/kg, 20 mg/kg and control group, were 70.4 ± 10.1 s, 41.47 ± 7.95 s, 70.58 ± 9.59 s and 59.06 ± 8.47 s respectively. Although, 5mg/kg reduced preference score, there was no significant difference in preferential score when all the CMC groups were compared to control.

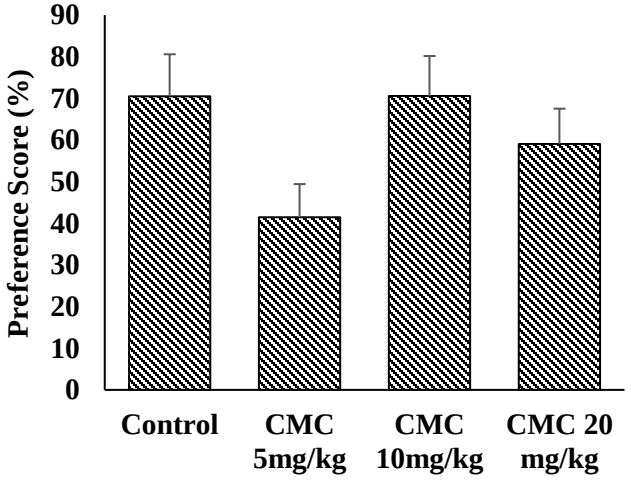

Figure: 1.
Effects of Carboxymethyl cellulose (CMS) administration on working memory in male Wistar rat (n = 5).

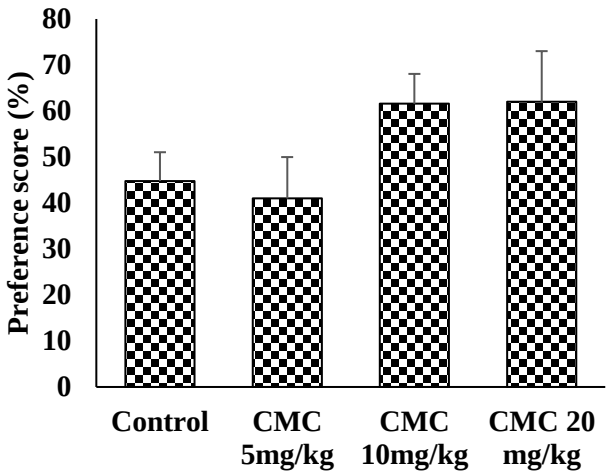

Figure: 2.
Effect of Carboxymethyl cellulose (CMS) administration on Long term memory in male Wistar rat (n = 5).

Long term memory

The Results obtained for long term memory is represented in figure 2. Preference score (working memory) for CMC 5 mg/kg, 10 mg/kg, 20 mg/kg and control group, were 44.77 ± 6.26, s 40.98 ± 9 s, 61.57 ± 6.49 s and 62 ±11.0 s respectively. There was no significant difference in preferential score when the CMC group were compared to the control.

Motor endurance

The results for motor endurance is represented in figure 3 for CMC 5mg/kg 10mg/kg, 20mg/kg and control group, were 15.38 ± 3.02 s, 63.02 ± 7.82 s, 50.08 ± 11.04 s and 36.56 ± 8.31 s respectively. There was a significant difference in 5mg/ml and 10mg/ml of the CMC groups when compared to the control.

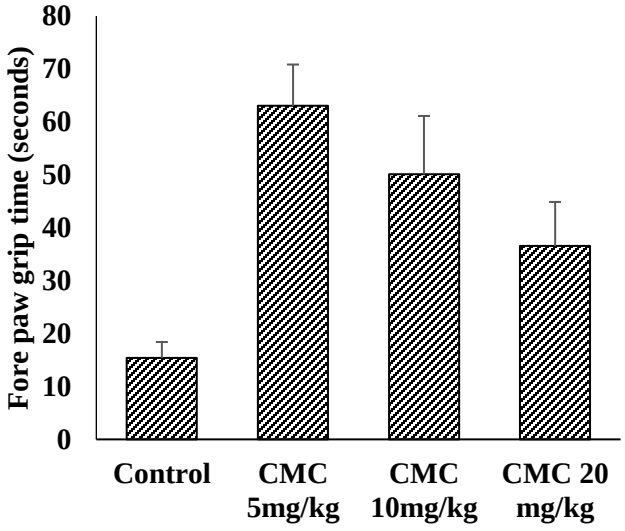

Figure: 3.
Effect of CMC administration on motor endurance in male Wistar rat (n = 5). * $P < 0.05$ significant compared to control.

DISCUSSION

Sodium carboxymethylcellulose (CMC) is a widely agent in many industries because it is reported to be non-toxic, non-immunogenic, non-pyrogenic, non-hemolytic, non-teratogenic, non-mutagenic, biodegradable, bioabsorbable and biocompatible (Du *et al*. 2015). In view of the uses of CMC, previous works have been done on the effects of CMC on its dietary effects as well its effects on blood cells (Brick, 1952; Anderson *et al*. 1986) but little or no work has been done on its effect on the nervous system or on its neurobehavioural effects. This study was performed to examine the effect of graded oral doses of CMC on cognitive and motor activities in male Wistar rats.

From the result obtained, the different concentration of CMC (5, 10 and 20 mg/kg) revealed no statistical significance alteration in preference score when compared to control animals, indicating little or no effect of CMC on learning and memory. This result agrees with the data reported by Muhammad *et al*. (2014).

The results obtained for motor endurance demonstrated significant difference when the effect of CMC was compared to that of the control. The most potent effect was observed at the lowest dose (5 mg/kg), this effect decreased with increase in the doses of CMC (10 mg/kg and 20 mg/kg). Taken together, the results demonstrated that CMC contributed to an increase in muscular endurance. This result disagrees with reports by Beck *et al*. (2006) and Wang *et al*. (2017) who used CMC as a placebo to evaluate supplementation with caffeine and creatinine respectively on motor endurance. In their studies, no improvement in motor endurance was recorded. The difference in their results and ours may be accounted for by: (1) The assumption by the previous authors that CMC did not affect motor endurance, hence it was used as a placebo, but in our study the hypothesis was that CMC may or may not have an effect on motor endurance. (2) The focus of their study was directed towards the supplement being investigated, while CMC served as a control (a reference agent). In our study, we set out to investigate neurobehavioural effect of CMC and compared its effects with a control group that received normal saline.

CMC is reported to be biologically, for that reason it is a common candidate as thickener and stabilizer in processed food as well as suitable vehicle in drug preparation and administration. Empirical evidence have shown that it may affect digestive functions, cause diarrhea at high doses, increased food and water intake and slightly elevated alanine phosphatase, alanine amino transferase and caecal enlargement (Bar *et al*. 1995). These may have no direct bearing or relevance on the present study but it substantiates the physiological activity of CMC.

Considering the results obtained in this study certain questions arose; is CMC a source of energy (although it is polymer of glucose) that stimulate storage of glycogen in the muscle causing an increase ability of muscle to contract for longer time without fatigue, despite the fact that CMC is indigestible in humans. What is the mechanism behind the increased concentration, causing decreased activity of CMC on muscular endurance? It is believed that certain protective mechanism in the brain like the blood brain barrier may prevent entry of CMC in the brain and render it inactive in the brain (Abbott *et al*. 2010; Campos-Bedolla *et al*. 2014). How will all these affect the metabolic effect of CMC on motor function? To our knowledge, there is currently no sufficient justification for the physiological effect of CMC, further studies on the underlying mechanism could provide more insight.

In conclusion, based on the result obtained in this study, it may be suggested that CMC significantly increased motor endurance and CMC did not significantly affect learning and memory.

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