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Long-term Consumption of *Capsicum annuum* (Chili Pepper) and Capsaicin Diets Elevates Anxiety but Improves Motor Coordination in CD-1 Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author EEO designed the study. Authors AUN and IEJ performed the statistical analysis and wrote the first draft of the manuscript. Authors AUN and SAB wrote the protocol. Authors UEO and EEO managed the analyses of the study. Author UEO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Chilli (*Capsicum annuum*), an extensively cultivated vegetable, is used to spice many dishes. It contains capsaicinoids, which give it a characteristic pungency. The most active and well known amongst these capsaicinoids is capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), which is neurogenic and may affect neuronal function.
Aim: It was hence, the aim of this present study to investigate the effects of long-term consumption of capsaicin and chilli pepper diets on anxiety and motor coordination.
Materials and Methods: Thirty male mice were randomly assigned into three groups of ten mice each, namely; control, pepper-diet (20% w/w) and capsaicin-diet (10%w/w) groups. Drinking water was allowed to all the animals *ad libitum.* The elevated plus maze and light-dark transition box were used to assess anxiety-related behaviour while the beam walking test was used to determine motor coordination in the mice.

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Results: The pepper and capsaicin diet-fed groups of mice had significantly shorter ($p<0.001$ and $p<0.01$ respectively) open arm durations compared to their control. While the head dips of the pepper group were not significantly different, that of the capsaicin group was significantly higher ($p<0.01$) compared to the control. The light chamber durations of both the pepper and capsaicin-diet fed mice were significantly shorter ($p<0.01$) compared to control. In the beam walking test to assess motor coordination, the frequency of foot slips for both pepper and capsaicin groups were significantly lower compared to control ($p<0.001$).
Conclusion: Long-term consumption of capsaicin and pepper diets increased anxiety but enhanced motor coordination in mice.

Keywords: Capsicum annuum; capsaicin; anxiety; motor coordination.

1. INTRODUCTION

All over the world, spices are used not only for their flavour but also for their medicinal properties. One of such spices is chilli pepper (*Capsicum annuum*), a vegetable belonging to the family *Solanaceae* [1] and used the world over for culinary purposes. It is the most widely and extensively cultivated vegetable amongst other species in the *Capsicum* genus [2]. Chillies originated from South and Central America [3]. They serve as additives in the preparation of various delicacies. Its flowers have off-white colour, while its stem is densely branched and can grow up to 60 cm tall. The plant is productive in warm and dry climate and can survive several seasons and grow into a large perennial shrub [4]. These chillies are berries which are usually red, green or yellow when ripe and widely consumed in Nigeria as well as other parts of the world.

The substances that give chillies their hot sensation and intensity when ingested or contacted are pungent chemical compounds collectively known as Capsaicinoids with Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) as the most abundant [5]. Exposure to the skin causes intense burning sensation while exposure to the eyes leads to intense tearing, conjunctivitis and blepharospasm [6].

The burning sensations associated with Capsaicin ingestion result from the activation of transient receptor potential, vanilloid 1 (TRPV1) located in the gut and other organs [7]. The stimulation of these TRPV1 receptors brings about the influx of sodium and calcium ion which results in the depolarization of nociceptive neurons, leading to the firing of action potentials and finally the sensation of spiciness [8].

Numerous health benefits are believed to emanate from Chilli pepper consumption [8]. Kempaiah et al. [9] reported that Capsaicin demonstrated protective effects against obesity and cholesterol by speeding up metabolism through stress hormone release. It is used as a topical agent in formulations against arthritis [10] and also in preparation of defensive sprays because of its irritant properties and ability to cause neurogenic inflammation (stinging sensation of hands, eyes and mouth) [11,12].

In our recent study, we observed that chronic consumption of chilli pepper and capsaicin containing diets impaired visuo-spatial learning and memory in CD-1 mice [13]. We also observed a decrease in food intake and an increase in water intake in mice. This decrease in food intake may be as a result of the effects of capsaicin which is believed to cause "a shift in substrate oxidation from carbohydrate to fat oxidation" [14].

Chilli pepper (*Capsicum annuum*) constitutes a part of the diet of many people around the world. It contains Capsaicin which is neurogenic [11] and so can affect neuronal components of the body [15], it is, therefore, conceivable that it may affect nervous function or behaviours such as anxiety and motor coordination. Hence, this present study investigated the comparative effects of long-term consumption of Capsaicin and Chillies on anxiety and motor coordination, using mice as an experimental model to ascertain if the effects obtained with pepper diet consumption can be attributed to Capsaicin.

2. MATERIALS AND METHODS

2.1 Preparation and Storage of Experimental Extracts

The half-washed basin of fresh red chilli pepper (*Capsicum annuum*) was procured from Watt Market in Calabar, Nigeria. It was washed and sun-dried for 4 days. The dried samples were then pulverised using an electric blender to

obtain a fine powder. The pepper powder was then stored in air-tight rubber container from which pepper diets were prepared. Forty (40) g of the dry pepper was extracted with 100 ml of 98% absolute ethyl alcohol for 40 mins in a continuous extraction apparatus (Soxhlet extractor). About 100 mls of the alcohol extract concentrate was filtered. The same procedure was repeated all over again but this time using distilled water as the extracting solvent. Both the ethanol and aqueous extracts were used for the phytochemical study while the aqueous extract was also administered to the mice in the pepper group for toxicity study. The extracts were stored in air-tight containers prior to their use.

Capsaicin (95% pure) was obtained from Wuxi Gorunjie natural-Pharma Co. Ltd, Jiangsu China. About 1 g of capsaicin was dissolved in 20 ml of normal saline (with each ml containing 50 mg of capsaicin) to form a stock solution for the toxicity study.

2.2 Animal Treatment

Thirty (30) male mice of CD-1 strain weighing between 22-34 g were used for the study. They were kept in a well-ventilated room under room temperature (25 ± 2°C), humidity of 85 ± 5% and 12/12 hours light/dark cycle and allowed one week for acclimatization to the research environment before the experiments. The mice were housed singly in metabolic cages where food and water intake were measured. They were randomly assigned into three groups, namely; control group that received normal rodent chow, pepper group that were fed 20% chilli pepper diet and capsaicin group that was given 10% capsaicin diet. Each group comprised 10 mice. Each mouse was allowed drinking water *ad libitum*. Treatment was for 28 days and within this period, their beddings, feed and water were hygienically handled and changed daily. After that, the assessment of anxiety and motor co-ordination in the mice was done.

2.3 Behavioural Protocol

2.3.1 The elevated plus maze

The elevated plus maze apparatus designed according to the description of Lister [16], and the test protocol adapted by Okon et al. [17] were used in this present study. This apparatus is used to assess the anxiety and fear levels of the mice. The test is based on the inborn aversion of rodents to open or bright illuminated spaces. The maze has two open arms (45 x 5 cm2) with 0.25 cm high edges and two closed arms (40 x 5 cm2) with 15 cm high walls radiating from a central square (5 x 5 cm). The open arms contain a slight edge (4 mm high) to prevent the mice from slipping and falling off the edge [18].

Prior to the test, the plus maze arms, surfaces and closed sides were cleaned with methylated spirit to eliminate olfactory clues and to remove fecal boll and urine. The mice were placed in the central square of the plus maze such that the mice faced an open arm away from the experimenter upon placement. Immediately after placement, a stop watch was started and the mice were allowed to explore the apparatus for 5 minutes. The test sessions were recorded and videotaped. Behaviours scored included open arm entry, open arm entry duration; head dip, rearing and stretch attend posture frequencies.

2.3.2 The light-dark transition box

The light and dark transition box checks for unconditioned anxiety. It is based on the clash between exploring in a new environment and aversion to bright light. T his box is divided into two compartments of unequal size as described by Costal et al. [19]. It is made up of plywood. The small compartment which is painted black has a measurement of 18 x 27 cm and constitutes 2/5 of the box. The larger (27 x 27 cm) compartment is painted white and makes up 3/5 of the box. Both compartments are linked by a door (7.5 x 7.5 cm) that is located at floor level in the centre of the wall separating the two compartments. The floor which is covered with Plexiglas is divided into 9 x 9 cm squares. The tests in this apparatus were conducted in a 2 x 5 m neurobehavioral laboratory which was lit by a 60 watts red lamp for background lighting. The mice were placed into the apparatus and allowed to explore for 5 minutes. The test sessions were recorded using video camera. Transitions, light chamber duration, stretch attend posture, rearing frequency, grooming duration was behaviours scored. The test room was dimly lit while the bright light chamber was particularly lit by a small 2watt energy bulb [20].

2.3.3 Beam walking

The beam walking assesses fine motor coordination and balance [21,22]. This test examines the ability of the mice to remain upright and to walk on an elevated and relatively narrow beam [22]. The beam has a length of 120 cm, a

width of 0.6 cm and is suspended about 60 cm above some foam pads.

The beam is marked at 5 cm and 1 cm intervals. It is composed of wood and is coated with black paint. The mouse was placed on one end of the beam. The trial was started after the mouse has secured its grip on the beam and lasted for approximately five minutes. The tests were videotaped for scoring. The parameters scored included the number of foot slips and falls.

2.4 Statistical Analysis

The data derived from the tests were analyzed by one-way analysis of variance (ANOVA) followed by post hoc student's Neuma-Keuls test using the computer software SPSS 2007 and Microsoft Excel 2007 for windows vista (Brain Series, China). Data were presented as mean ± SEM (Standard error of the mean) and p value less than 0.05 was considered statistically significant

3. RESULTS

3.1 Effects of Consumption of Chilli Pepper (*Capsicum annuum*) and Capsaicin on Fear and Anxiety in the Elevated Plus Maze

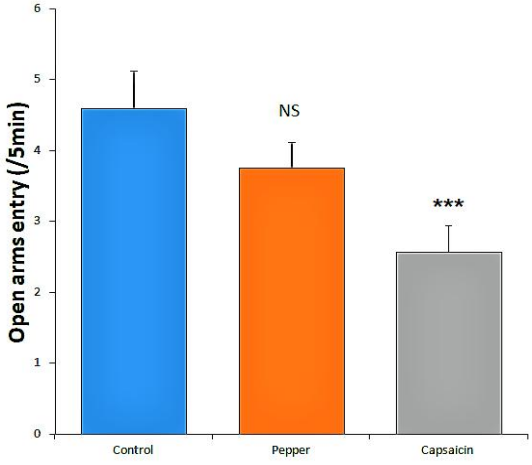
The open arm entry frequency of the pepper group was not significantly different compared to the control, whereas that of the capsaicin group was significantly lower ($p<0.001$) compared to the control (Fig. 1A). Both the pepper and capsaicin groups had significantly shorter ($p<0.001$ and $p<0.01$ respectively) open arm durations compared to the control (Fig. 1B).

There was no significant difference in the head dips frequency of the pepper-diet fed mice compared to the control (Fig. 1C). However, that of the capsaicin-diet fed mice was significantly higher compared to the control ($p<0.01$). While the stretch attends posture of the pepper group was not significantly different compared to control, that of the capsaicin group was significantly higher compared to both the control and pepper groups ($p<0.001$) (Fig. 1D).

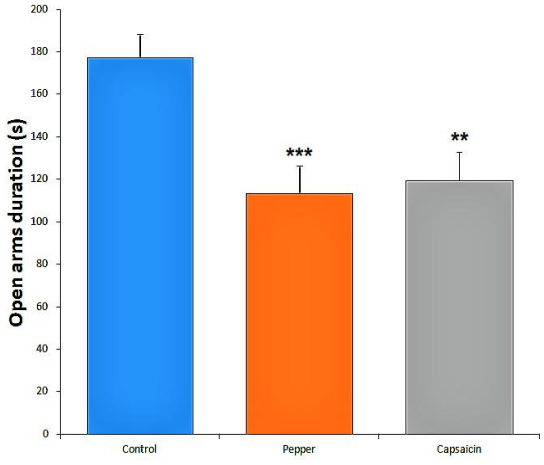
The frequencies of rearing of both the pepper and capsaicin groups were significantly higher compared to control ($p<0.01$ and $p<0.001$ respectively) (Fig. 1E). The value for the capsaicin group was significantly higher ($p<0.01$) compared to the pepper group.

3.2 Effects of Consumption of Chilli Pepper (*Capsicum annuum*) and Capsaicin on Fear and Anxiety in the Light-dark Box Transition

The rearing frequencies of both the pepper and capsaicin-diet fed mice were significantly higher ($p<0.001$) compared to control. However, rearing frequency of the capsaicin group was significantly higher ($p<0.001$) compared to the pepper group (Fig. 2A). The transition frequency of the pepper group was not significantly



A



B

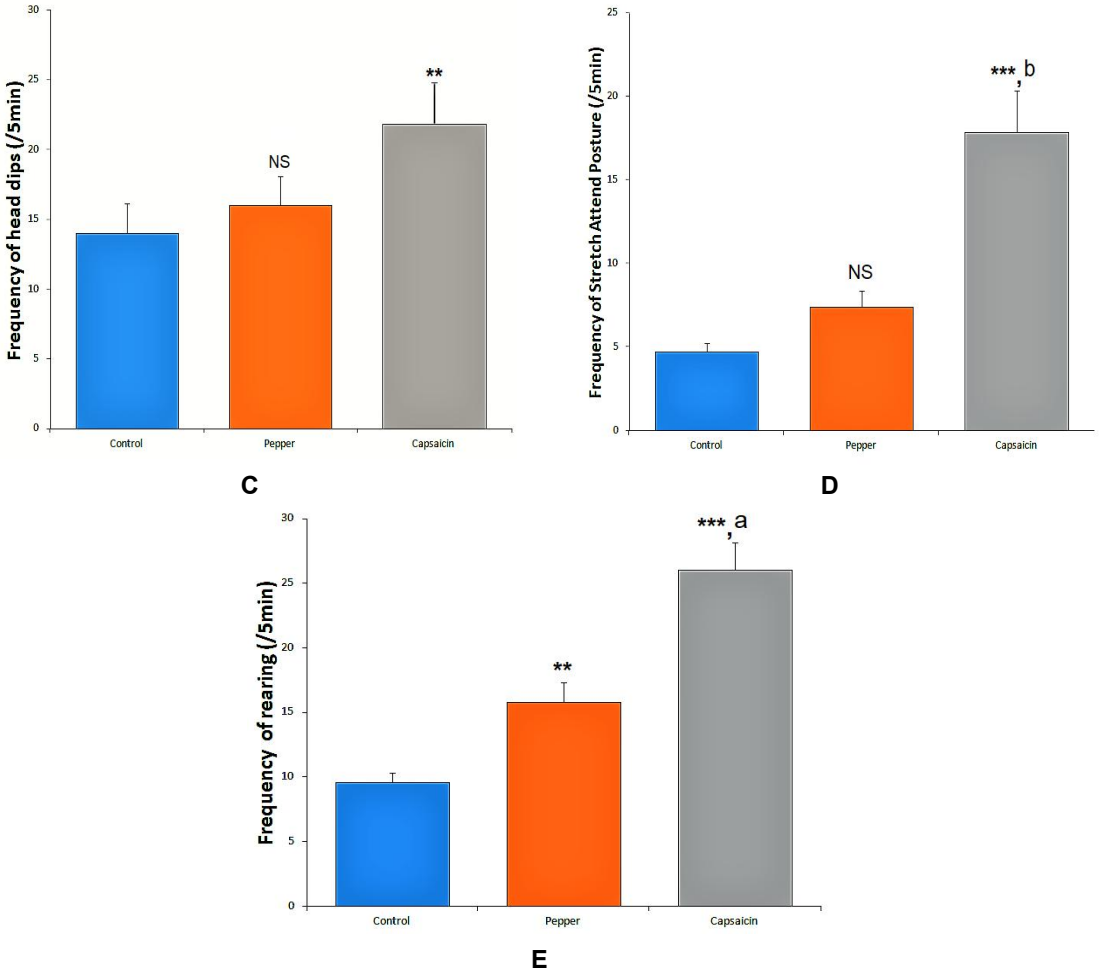


Fig. 1. Comparison of (A) Open arm entry frequency (B) Open arm duration (C) Head dip frequency (D) Stretch attend posture frequency (E) Grooming frequency in the elevated plus maze test of the different experimental groups

Values are mean ± SEM, n= 10.
*NS= Not significant, **=p<0.01, ***=p<0.001 vs control;*
a=p<0.01, b=p<0.001 vs pepper

different compared to the control. The value for the capsaicin group was significantly lower compared to both the control and pepper groups ($p<0.001$ and $p<0.01$ respectively) (Fig. 2B).

The light chamber duration of both the pepper and capsaicin groups were significantly shorter compared to the control ($p<0.01$) (Fig. 2C). Both the pepper and capsaicin groups had significantly longer ($p<0.05$) grooming durations compared to the control (Fig. 2D).

The stretch attend postures of both the pepper and capsaicin group were significantly higher ($p<0.05$ and $p<0.001$) compared to the control (Fig. 2E). However, that of capsaicin group was significantly higher ($p<0.01$) compared to the pepper group.

3.3 Effects of Consumption of Chilli Pepper (*Capsicum annuum*) and Capsaicin on Motor Coordination in the Beam Walking Test

The foot slips of both the capsaicin and pepper groups were significantly lower compared to the control ($p<0.001$) (Fig. 3A). Also, the number of falls of both the capsaicin and pepper groups were significantly lower ($p<0.001$ and $p<0.01$ respectively) compared to control (Fig. 3B).

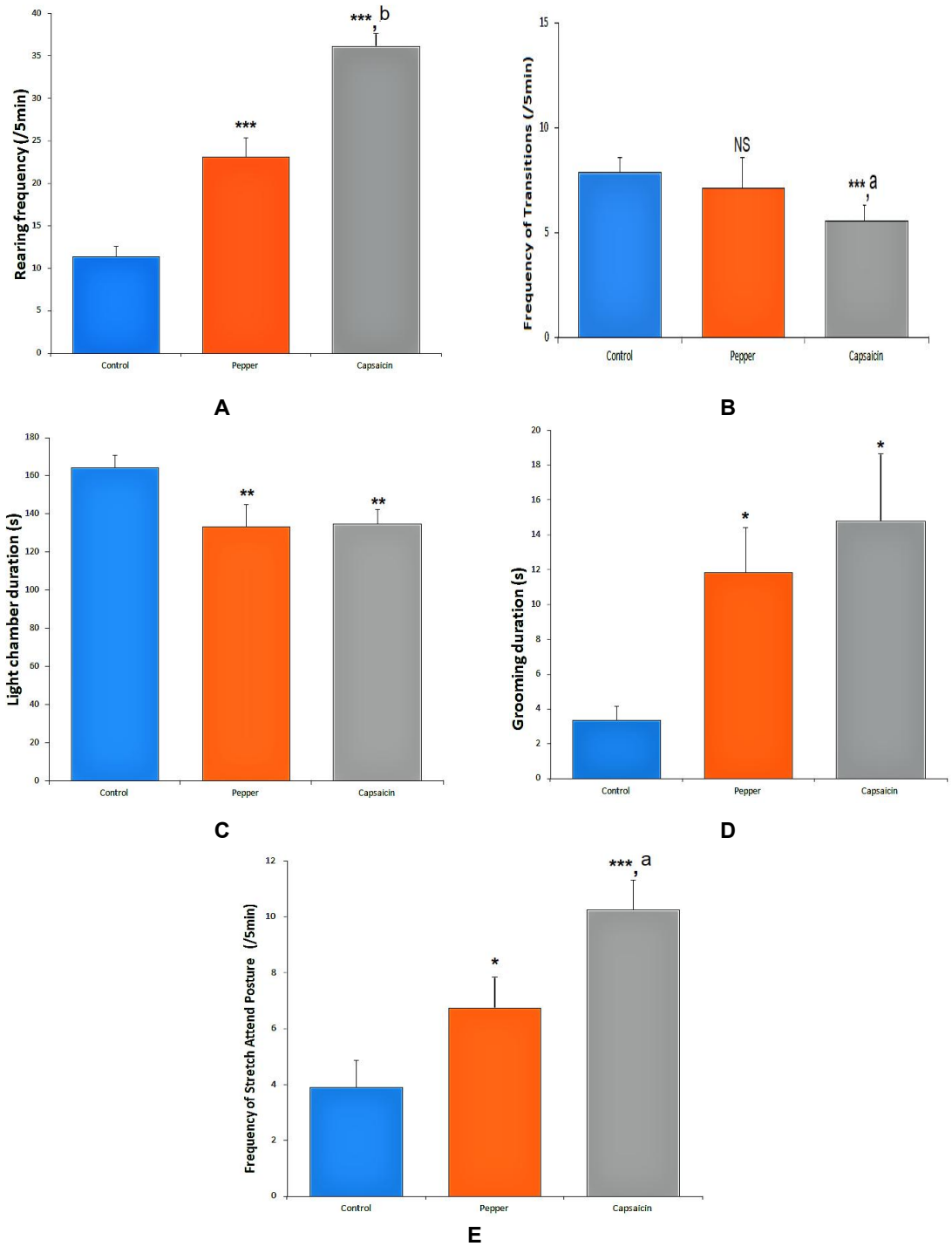


Fig. 2. Comparison of (A) rearing frequency (B) transition frequency (C) light chamber duration (D) grooming duration (E) stretch attend posture in the Light-Dark transition box test of the different experimental groups

Values are mean ± SEM, n= 10.
*NS= Not significant, *=p<0.05, **=p<0.01, ***=p<0.001 vs control;*
a=p<0.01, b=p<0.001 vs pepper

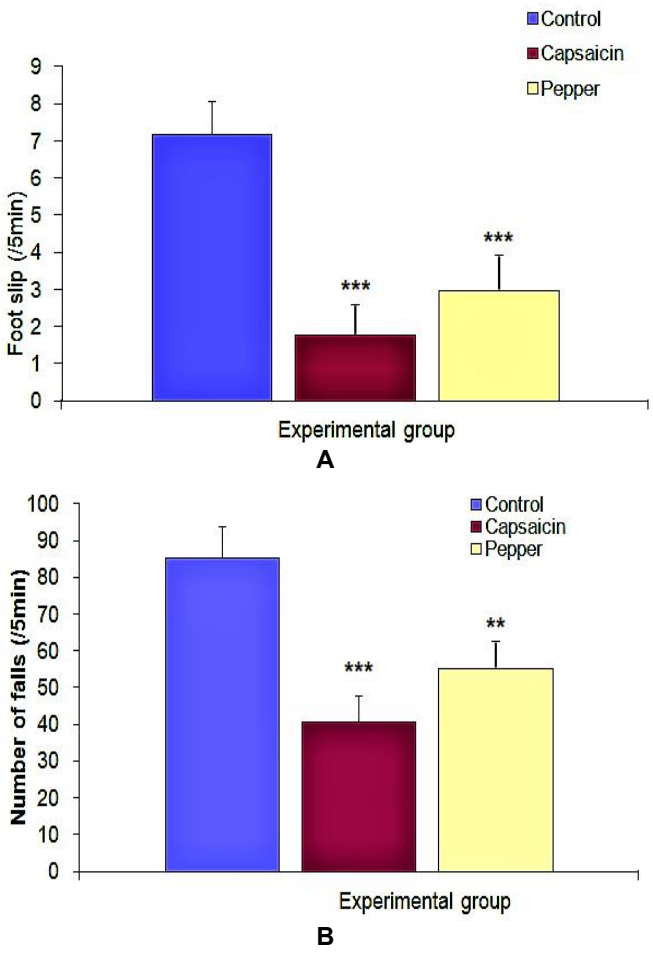


Fig. 3. Comparison of (A) Foot slips (B) Number of falls in the Beam Walking test of the different experimental groups

Values are mean ± SEM, n= 10.
***=p<0.01, ***=p<0.001 vs control*

4. DISCUSSION

In our recent study, we reported that long term consumption of chilli pepper and capsaicin diets impaired visuo-spatial learning and memory in CD-1 mice [13]. Food intake was not altered but water intake was higher in mice fed pepper and capsaicin diets. This present study elucidates the effects of these two forms of diets on other nervous function parameters namely; anxiety and motor coordination. The ED_{50} used for this present study was derived from the LD_{50} of our previous study [13].

Following the consumption of pepper and capsaicin diets, the pepper and capsaicin groups had lower open arm entry frequency and duration in the elevated plus maze test. Since fearful mice tend to avoid open areas (especially when they are brightly lit), favouring darker and more enclosed spaces [23], these results imply that chilli pepper and capsaicin caused increase in anxiety in the mice.

Increase in risk assessment behaviours such as head dipping and stretch attend posture indicate increased anxiety levels [24]. The results showed that though not significant, the head dips and stretch attend postures of the pepper group were slightly higher than control. On the other hand, the head dips and stretch attend postures of the capsaicin group was significantly higher than the control. These results showed that both pepper and capsaicin increased anxiety in the mice, but the anxiogenic effects of capsaicin were greater.

The rearing frequencies of both the capsaicin and pepper groups were significantly higher than control. This means the anxiety levels of mice fed pepper and capsaicin diets were high. This is because, the higher the rearing frequency, the higher the anxiety level.

In the light-dark transition box tests, most mice naturally demonstrate a preference for the dark chamber. Mice with less level of anxiety tend to venture more into the light compartment. The pepper and capsaicin-diet fed mice spent less time in the light chamber than the control. This implies that they were more anxious.

From the results, the rearing frequencies, grooming durations and stretch attend postures of both the pepper and capsaicin-diet fed mice were higher than the control. These showed that pepper and capsaicin increased anxiety in the mice, because the higher the grooming duration, rearing frequency and stretch attend posture, the higher the levels of anxiety.

Mice exhibiting higher levels of anxiety related behaviours make fewer transitions between the brightly illuminated area and the dark compartment. Pepper and capsaicin significantly reduced the number of transitions of the mice between the light and dark chambers. These behaviours confirm the anxiogenic tendencies of long term administration of pepper and capsaicin. However, its mechanism of action has not been ascertained. This is in keeping with the study of Choi et al. [25]. Hakimizadeh et al. [26] also reported that direct injection of capsaicin in the hippocampus induces anxiety-like behaviours, but the report of Santos et al. [27] was on the contrary.

Capsaicin and pepper-diet fed mice had significantly reduced foot slips and falls compared to control and these typify improvement in their motor coordination because the lower the frequency of foot slips and number of falls, the better coordinated the animal was. Capsaicin, as the most abundant and commonly occurring capsaicinoid might have achieved this feat (improvement of motor coordination) by aiding in the integration of proprioceptive information with neural processes (TRPV1) in the spinal cord and in the brain (specifically cerebellum) [28].

5. CONCLUSION

Long-term consumption of both chilli pepper and capsaicin diets increased anxiety-related behaviours but enhanced motor coordination. Therefore, it is likely that capsaicin which is an active and stable alkaloid in chillies may be responsible for the anxiogenic and enhanced motor coordination potentials observed in mice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 8523, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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