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Chapter

The Effects of Consumption of *Capsicum* on Some Neurobehavioural Parameters

Eme Efiom Osim, Anyauba Nmaju, Azubuike Nwankwo, Udemeobong Okon and Joshua Iwasam

Abstract

Capsicum annum, an extensively cultivated vegetable, is commonly used to spice many dishes prepared in several parts of the world. 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 functions. Therefore, our study investigated the effects of consumption of chilli pepper and capsaicin diets on neurobehaviour of CD-1 mice. The neurobehavioural parameters assessed were anxiety, motor coordination, pain, social behaviour, learning and memory. The animals were randomly assigned into three groups of 10 mice each, namely, control, pepper-diet (20% w/w), and capsaicin-diet (10% w/w) groups. Their learning and memory abilities were assessed through their ability to locate the hidden platform model of Morris water maze apparatus. The elevated plus maze and light-dark transition box were used to assess anxiety-related behaviour, while the beam walking test and nesting behaviour were used to determine motor coordination and social behaviour, respectively. Tail immersion, hot plate, and formalin tests were conducted to assess pain perception. Consumption of the chilli pepper and capsaicin diets decreased pain perception, increased anxiety, and impaired learning and memory but enhanced social behaviour and motor coordination in mice.

Keywords: *Capsicum annum*, capsaicin, pain, social behaviour, anxiety, motor coordination, learning and memory

1. Introduction

Spices are used all over the world not only for their flavours but also for their medicinal properties [1]. One of such spices is chilli pepper. Chilli pepper or chillies are vegetables used world over for culinary purposes and belong to the family Solanaceae [2].

These peppers are widely used as spices in food industry and in a broad variety of medicinal applications worldwide [3]. These spices are remarkable sources of antioxidant compounds including phenolic compounds and flavonoids, of which their consumption has potential health benefits due to their activity as free radical scavengers and may also help prevent inflammatory diseases and pathologies associated with oxidative damage such as atherosclerosis and Alzheimer's disease [3].

Chilli peppers are called *Ntokon* in Efik, *Osé* in Ibo and *Brukunu* in Hausa languages in Nigeria. They are usually red or green in colour. It is the most commonly consumed pepper in Nigeria.

The substances that give chillies their hot sensation and intensity when ingested or contacted are pungent chemical compounds collectively known as Capsaicinoids with Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) as the most abundant [4]. Exposure to the skin causes intense burning sensation while exposure to the eyes leads to intense tearing, conjunctivitis and blepharospasm [5].

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 [6]. The stimulation of these TRPV1 receptors brings about the influx of sodium and calcium ions which results in the depolarization of nociceptive neurons, leading to the firing of action potentials and finally the sensation of spiciness [7]. Capsaicin is an alkaloid which is highly volatile, odourless, colourless and hydrophobic [8]. It structurally, belongs to a group of chemicals known as vanilloids, having a vanillyl (methylcatechol) head group (A-region) and an aliphatic tail (hydrophobic—C-region) linked by a central amide bond (B-region). This combination of these regions is accountable for the pharmacological activities of capsaicin [8].

Numerous health benefits are believed to emanate from Chilli pepper consumption [7]. 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].

Capsicum annum contains capsaicin which is neurogenic [11] and therefore, can affect neuronal activities in the body [13]. It is therefore conceivable that it may affect some nervous function parameters such as pain, social behaviour, anxiety, motor coordination, learning and memory. Hence, we present the report of our study that investigated the comparative effects of long-term consumption of capsaicin and chillies on the aforementioned neurobehavioural parameters using mice as experimental models to ascertain whether the effects obtained with pepper-diet consumption can be attributed to capsaicin.

2. Materials and methods

2.1 Preparation and storage of experimental diets

Half-washed basin of fresh red chilli pepper (*Capsicum annum*) was procured from a local market in Nigeria. It was washed and sun-dried for 4 days. The dried samples were then pulverised using electric blender to obtain a fine powder. The pepper powder was then stored in air-tight rubber container from which pepper diets were prepared.

Capsaicin (95% pure) was obtained from Wuxi Gorunjie natural-Pharma Co. Ltd., Jiangsu China.

2.2 Animal treatment

Thirty (30) male mice of CD-1 strain weighing between 22 and 34 g were used for the study. They were kept in a well-ventilated room under room temperature (25 ± 2°C), humidity of 8 ± 5% and 12/12 h light/dark cycle and allowed 1 week for acclimatisation to the research environment before the experiments. 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 were given 10% capsaicin diet. Each group comprised 10 mice. Each mouse was allowed drinking water *ad libitum*. This treatment was done for 28 days and within this period, their beddings, feed and water were hygienically handled and changed daily.

2.3 Approval for animal use

Approval for this study was obtained from the Faculty Animal Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar with Protocol number 014PY20314.

2.4 Behavioural protocol

2.4.1 Assessment of anxiety levels

2.4.1.1 The elevated plus maze

The elevated plus maze apparatus designed according to the description of Lister [14], and the test protocol adapted by Okon et al. [15] 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 × 5 cm^2) with 0.25 cm high edges and two closed arms (40 × 5 cm^2) with 15 cm high walls radiating from a central square (5 × 5 cm). The open arms contain a slight edge (4 mm high) to prevent the mice from slipping and falling off the edge [16].

Prior to the test, the plus maze arms, surfaces and closed sides were cleaned with methylated spirit to eliminate olfactory clues and to remove faecal 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 min. 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.

2.4.2 Assessment of motor coordination

2.4.2.1 Beam walking

The beam walking assesses fine motor coordination and balance [17]. This test examines the ability of the mice to remain upright and to walk on an elevated and relatively narrow beam [17]. The beam has a length of 120 cm, a width of 0.6 cm and is suspended about 60 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 5 min. The tests were videotaped for scoring. The parameters scored included the number of foot slips and falls.

2.4.3 Assessment of pain perception

2.4.3.1 Tail immersion test

This test used by Ramabadran et al. [18] assesses the basic pain response in mice to thermal stimuli. This test measures spinally driven aspects of pain. Here, the

animals were immobilised using tube restrainer (which also allowed free movement of the tail). The distal half of each mouse tail was immersed in water bath (at 50°C) contained in a beaker with 20 s time-out. The duration of time taken for the mice to flick its tail away from the heat of the hot water was measured in seconds. This is known as latency of flick [19].

2.4.3.2 Hot plate test

This is a test of thermal nociception, model of short duration stimuli [20]. Each mouse was exposed to a hot surface within a confined glass cage (whose temperature was maintained at 55 ± 0.5°C) for maximum duration of 30 s [21]. The time it took for each mouse to start licking its foot pad was recorded. Higher frequency and duration of paw lick indicate higher pain perception. The time taken for it to jump (latency of jump) was also recorded. The longer the latency of jump, the less pain it felt. These behaviours are the most common measures of pain threshold and are considered supra-spinally integrated [20].

2.4.4 Assessment of social behaviour

Nesting behaviour test used by Bender et al. [22] and Deacon [23] as an assay for social behaviour was used in this present study. The test was conducted in individual home cages. In total, 1 h before giving the mice nestling materials, all enrichment objects were removed from the home cages of the mice. About 3.0 g of nestling material was supplied to each mouse in its home cage and allowed for 24 h after which the nests were assessed using the rating scale supplied by Deacon [23] (**Table 1**). This assessment was based on what was seen in the home cages of the mice. Extreme care was taken while observation was carried out, as causing panic to a mouse could result in the destruction of the nest that was built.

Rating	Requirements
1	Nestlet not noticeably touched (90% or more intact)
2	Nestlet partially torn (50–90% intact)
3	Nestlet mostly shredded, often no identifiable nest site, 50–90% shredded, also, less than 50% remains intact, but less than 90% is within a quarter of the cage floor (i.e., not gathered into a nest site but spread throughout cage)
4	An identifiable, but flat nest, more than 90% of the nestlet is torn, the nest is uneven, material is gathered into a nest within a quarter of the cage floor, but the nest is flat with walls higher than mouse body height for less than 50% of its circumference
5	A (near) perfect nest, more than 90% of the nestlet is torn, nest is fairly even, the nest is a crater, with walls higher than the mouse body for more than 50% of its circumference

Source: Deacon [23].

Table 1.
Nesting behaviour rating scale.

2.4.5 Assessment of learning and memory

The Morris water maze developed by Morris [24] for assessing visuospatial learning and memory was used in this study. It was made of a circular polypropylene pool which was divided into four quadrants: Northwest, Northeast, Southwest and Southeast. It measured about 85 cm and 20 cm in diameter and

depth respectively. The pool was filled to depth of 14 cm with water. The water was left to sit overnight in order to achieve room temperature (about 26 ± 2°C) and made opaque with the addition of milk to ensure camouflage of the escape platform. The platform was submerged to about 1 cm below the water surface. The pool was located in the laboratory with posters of diagrams hung on the walls to act as visual cues. During testing, the room was dimly lit with diffuse white light. The performance of the animals in the maze was recorded using a camcorder.

Testing in the Morris water maze lasted for 8 days. The first 3 days were for acquisition training with an invisible platform. The next 3 days were for reversal training with the hidden platform in an opposite quadrant. On the seventh day, a probe trial was conducted with no escape platform. On day eight, 4 trials were conducted with a visible platform. Sixty (60) seconds were allocated for each mouse to locate the platform during each trial. Mice which were unable to locate the platform were guided to the position of the platform. The timer was stopped when the mice located the platform within the 60 s. The time it took the mice to locate the platform was recorded as swim latency. After each trial, mice were placed in cages with shredded paper towel beddings to make them dry easily and a heating lamp was also provided to prevent animals from developing hypothermia.

2.5 Statistical analysis

The data derived from the tests were analysed by one-way analysis of variance (ANOVA) followed by post hoc student's Neuma 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 pepper (*Capsicum annum*) 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 (**Figure 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 (**Figure 1b**).

There was no significant difference in the head dips frequency of the pepper-diet fed mice compared to the control (**Figure 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 bot and pepper groups ($p < 0.001$) (**Figure 1d**).

3.2 Effects of consumption of chilli pepper (*Capsicum annum*) 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 ($p < 0.001$) (**Figure 2A**). 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 (**Figure 2B**).

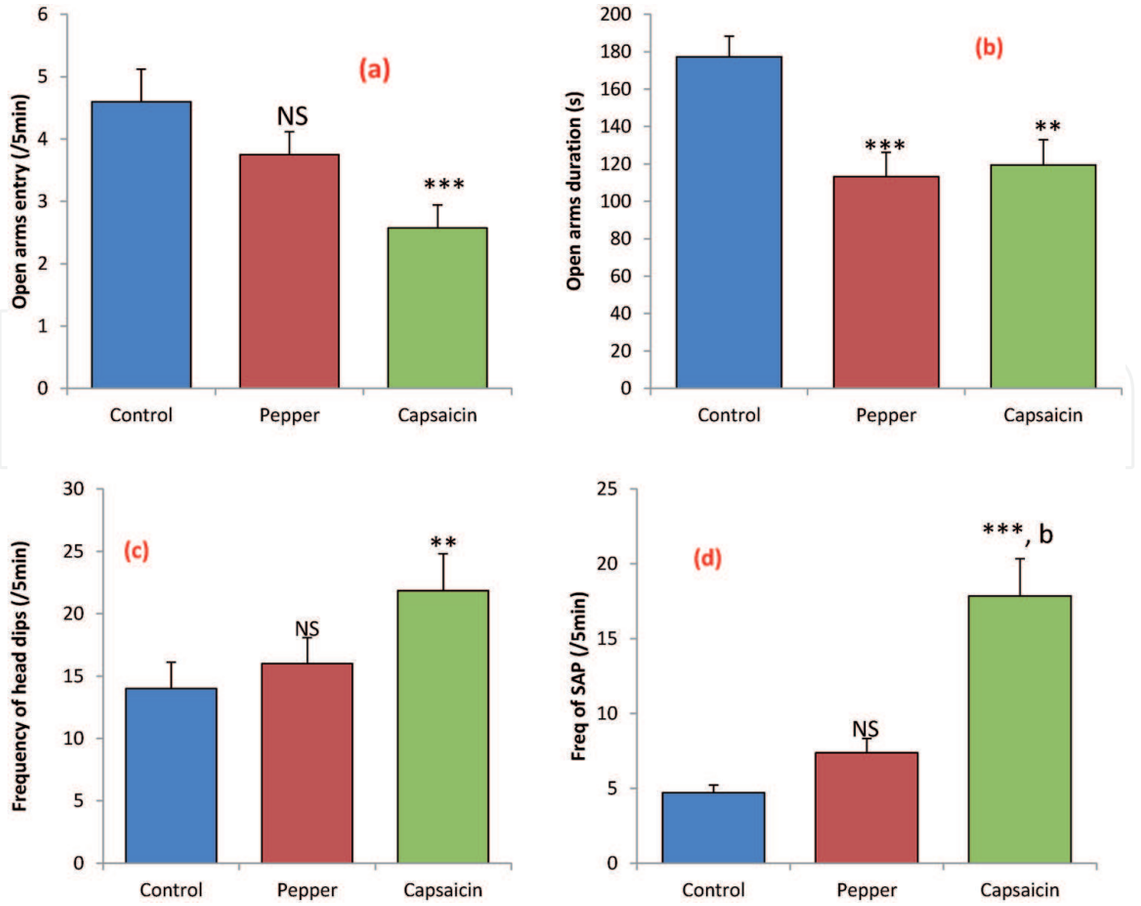


Figure 1.
*Comparison of (a) open arm entry frequency, (b) open arm duration, (c) head dip frequency and (d) stretch attend posture in the elevated plus maze test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. NS = not significant, ** = p < 0.01, *** = p < 0.001 vs control; b = p < 0.001 vs pepper group.*

3.3 Effects of consumption of chilli pepper (*Capsicum annum*) and capsaicin on pain

3.3.1 Tail flick test

During the first test, the latency of tail flick of both the pepper and capsaicin groups were significantly longer ($p < 0.01$ and $p < 0.001$, respectively) compared to control. The value for the capsaicin group was significantly longer ($p < 0.01$) compared to that of pepper group (**Figure 3**).

3.3.2 Hot plate test

The latency of jump of both pepper and capsaicin groups were significantly longer compared to control ($p < 0.01$ and $p < 0.001$, respectively). Meanwhile, the latency of jump of the capsaicin group was significantly longer compared to the pepper group ($p < 0.001$) (**Figure 4a**). While the frequency of hind paw lick of the pepper group was not significantly different compared to the control, the value for capsaicin group was significantly lower compared to both control and pepper groups ($p < 0.001$ and $p < 0.05$, respectively) (**Figure 4b**). Pepper group had a paw lick duration that was not significantly different compared to control. However, the value for capsaicin group was significantly shorter ($p < 0.01$) compared to control (**Figure 4c**).

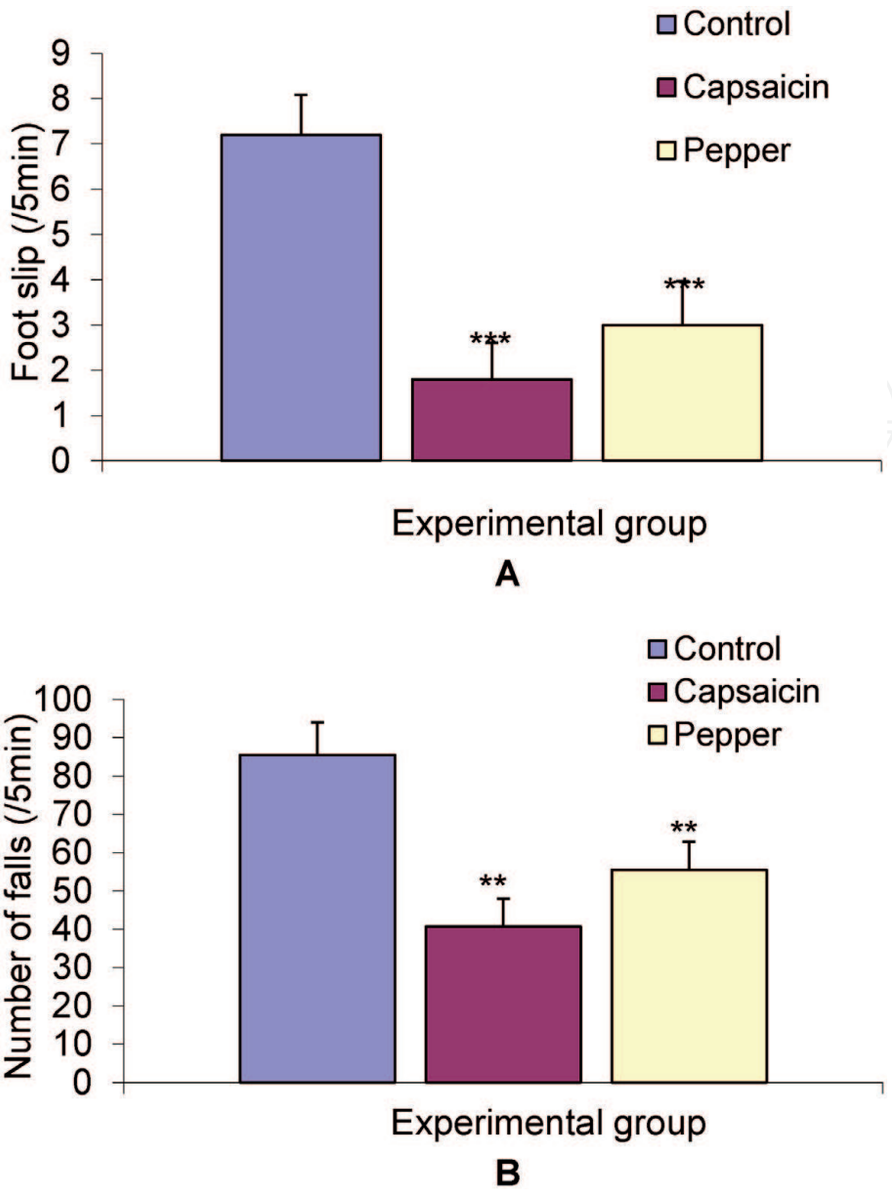


Figure 2.
*Comparison of (A) foot slips and (B) number of falls in the beam walking test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. ** = p < 0.01, *** = p < 0.001 vs control.*

3.4 Effects of consumption of chilli pepper (*Capsicum annum*) and capsaicin on social behaviour in the nesting behaviour test

The nesting score of the capsaicin group was significantly higher ($p < 0.01$) compared to control. The value for the pepper group appeared higher than control but was not significant. However, it was significantly lower ($p < 0.05$) compared to that of the capsaicin group (**Figure 5**).

3.5 Comparison of swim latency in the Morris water maze test for learning

During the acquisition training, the capsaicin group had a significant longer ($p < 0.001$) swim latency on days 2 and 3 compared to control (**Figure 6A**). The swim latency during the reversal training was significantly longer in both capsaicin and pepper groups on day 1 compared to control ($p < 0.001$) but not different on days 2 and 3 (**Figure 6B**).

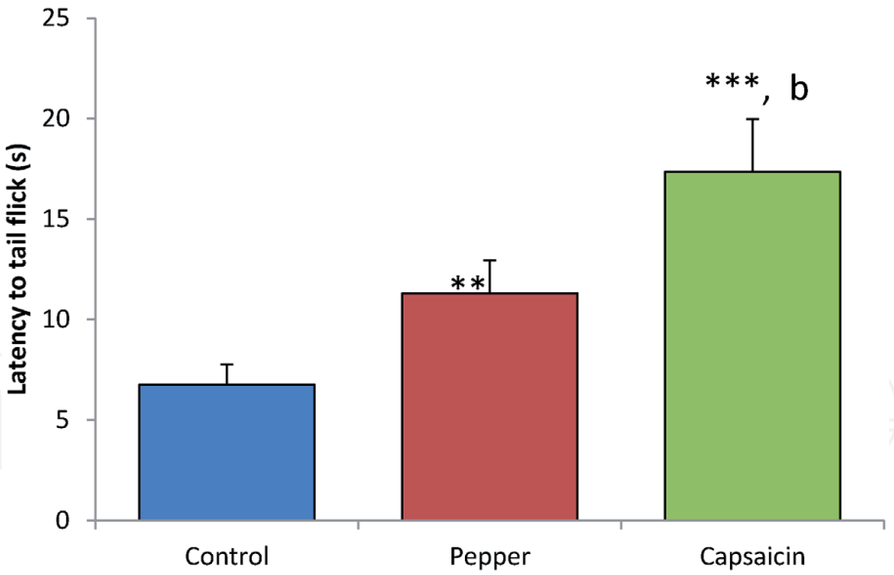


Figure 3.
*Comparison of latency of tail flick in the tail immersion test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. ** = p < 0.01, *** = p < 0.001 vs control; b = p < 0.01 vs pepper group.*

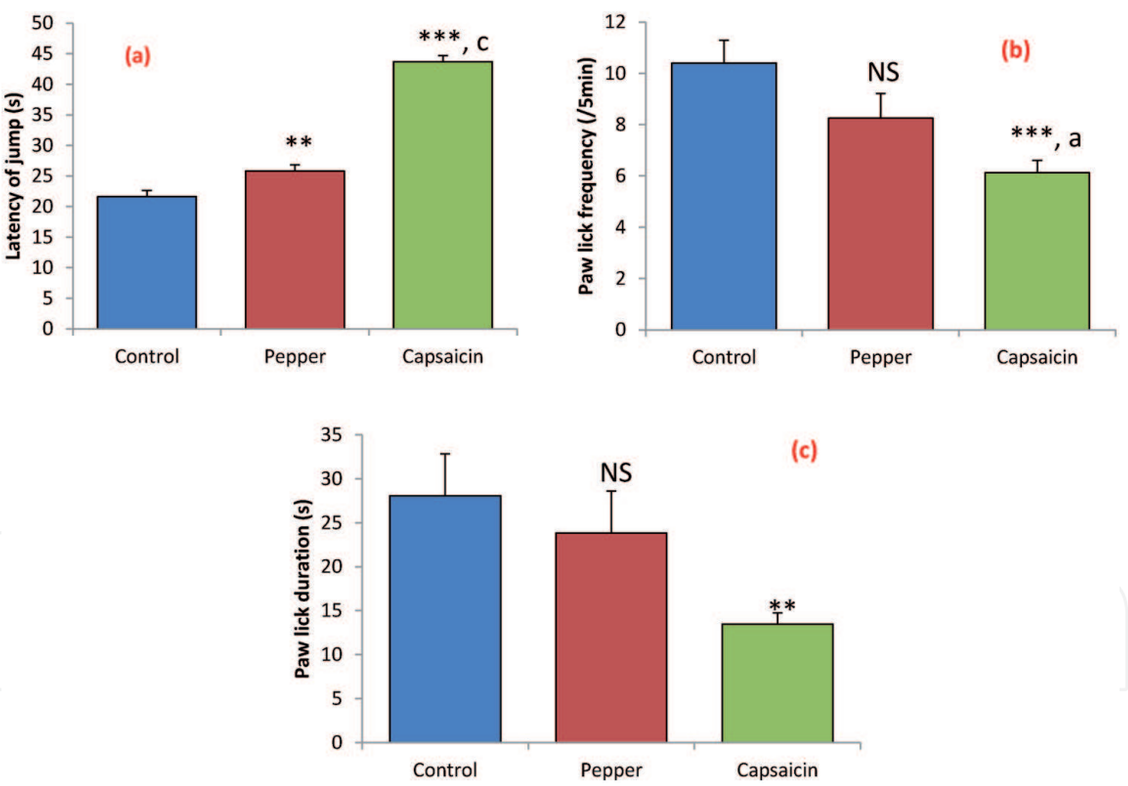


Figure 4.
*Comparison of (a) latency of jump, (b) paw lick frequency and (c) paw lick duration in the hot plate test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. N = not significant, ** = p < 0.01, *** = p < 0.001 vs control; a = p < 0.05, c = p < 0.001 vs pepper group.*

3.6 Comparison of quadrant duration in the Morris water maze test for memory in the different experimental groups

In the probe trial, the pepper and capsaicin groups showed a significantly shorter quadrant duration compared to control ($p < 0.001$ and $p < 0.01$ respectively) (**Figure 7**).

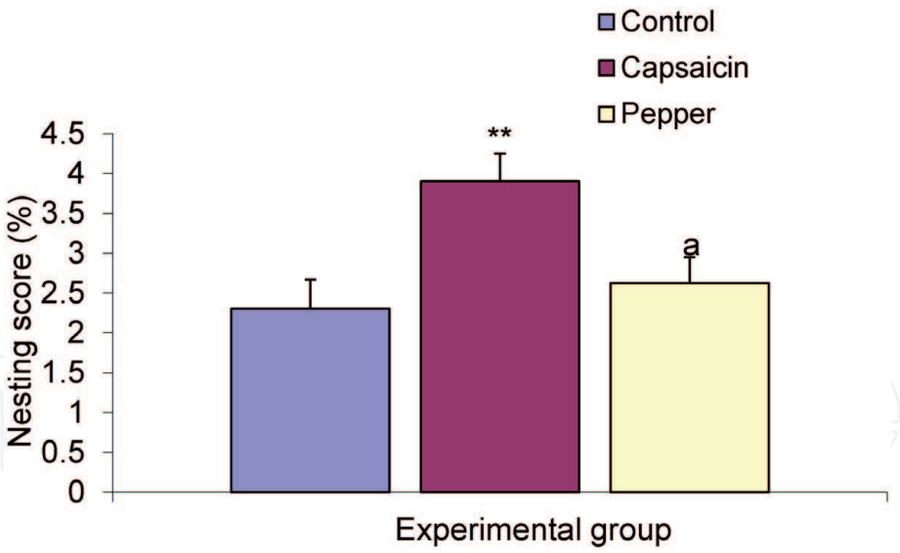


Figure 5.
*Comparison of the nesting score of the different experimental groups. Values are expressed as mean ± SEM, n =10. ** = p < 0.01 vs control; a = p < 0.05 vs capsaicin group.*

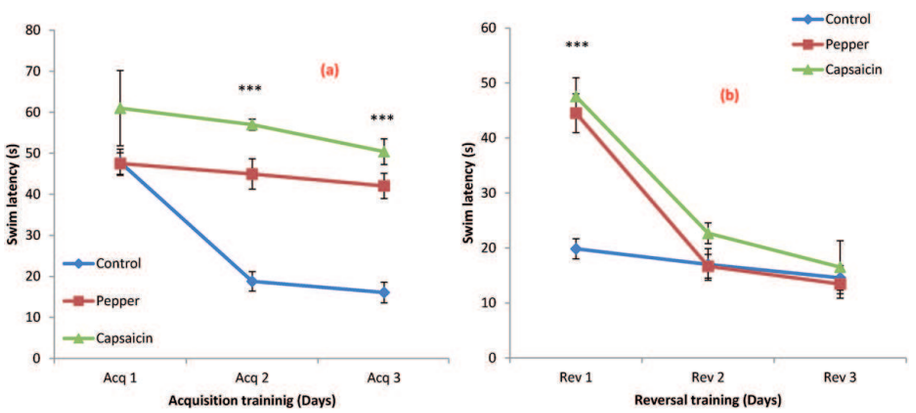


Figure 6.
*Comparison of the swim latencies of the different experimental groups in (a) acquisition and (b) reversal trainings. Values are expressed as mean ± SEM, n = 10. *** = p < 0.001 vs control.*

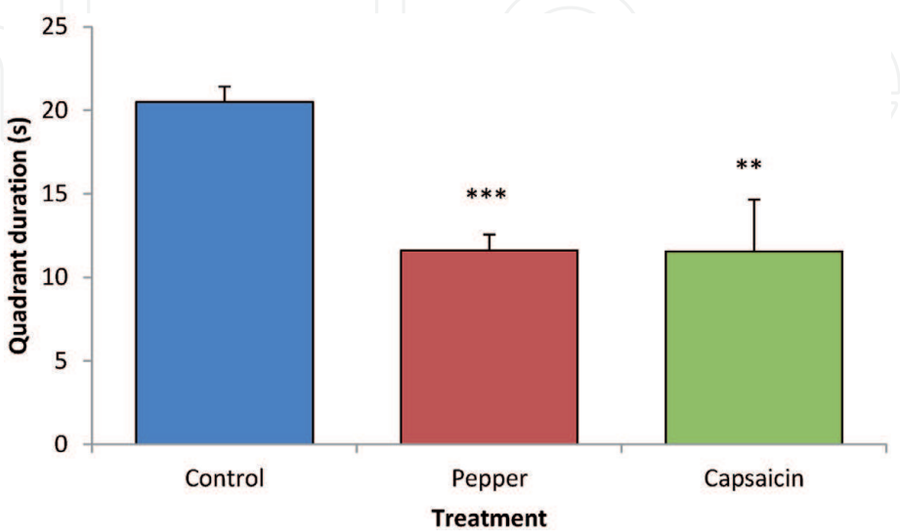


Figure 7.
*Comparison of quadrant duration in Morris water maze test of the different experimental groups Values are expressed mean ± SEM, n = 10. ** = p < 0.01, *** = p < 0.001 vs control.*

4. Discussion

4.1 Anxiety

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 [25], these results imply that chilli pepper and capsaicin caused increase in anxiety in the mice [26].

Increase in risk assessment behaviours such as head dipping and stretch attend posture indicate increased anxiety levels [27]. 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 [26].

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 agreement with the study of Choi et al. [28]. Hakimizadeh et al. [29] also reported that direct injection of capsaicin in the hippocampus induces anxiety-like behaviours, but the report of Santos et al. [30] was on the contrary.

4.2 Motor coordination

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 [26]. 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) [31].

4.3 Pain

Tail-flick response of a mouse to thermal stimuli is believed to be a spinally mediated reflex behaviour [32]. From the results of our study, the latencies of tail flick of both the pepper and capsaicin-diet fed mice were significantly longer than those of control in the immersion test. Also, the tail-flick latency of the capsaicin group was longer than that of the pepper group. These results imply that pepper or capsaicin diet increased the pain threshold of the mice leading to decreased pain sensitivity. These results point to a more effective analgesic potential in capsaicin than pepper [33].

In the hot plate test, when an animal perceives pain, it attempts to jump away from the object that elicited the painful stimuli. This supra-spinally controlled behaviour (latency of jump) models an escape behaviour. The latencies of jump of both pepper and capsaicin groups were significantly longer than the control. Also, the latency of jump of the capsaicin group was significantly longer than that of pepper group. These results depicted that both capsaicin and pepper exhibited anti-nociceptive tendencies because it took a long time for mice fed such diets to experience pain compared to control. These results further confirm the analgesic potentials of pepper and capsaicin [33].

Capsaicin may have acted by initially activating heat sensitive TRPV1 receptors which induce pain. Repeated and prolonged exposure to capsaicin might have resulted in the reduction of responsiveness of the receptors and ion channels, thus

leading to the "defunctionalization" of nociceptor fibres as reported by Anand and Bley [34]. Therapeutic uses of capsaicin for pain treatment were reported; Evangelista [35] and Chung and Campbell [36] reported that capsaicin can be used in treatment of neuropathic pain. Another study reported the analgesic effects of topically applied capsaicin [37]. These reports are in keeping with the results of the present study.

4.4 Social behaviour

The nesting score is an assessment of social behaviour. This nesting behaviour is a reflection of the social behaviour in mice. Nest building in mice correlates to organised behaviour in humans and is very distinct from the findings reported in a research by Alleva et al. [38], where they reported aggressive behaviour in mice treated with capsaicin. A poor performance in the nesting task may indicate impairment in social relationship in mice and likelihood of the presence of autistic behaviour. The nesting score of the capsaicin group was significantly higher compared to that of control. The value for pepper group appeared higher but was not significantly higher than the control. This result indicates enhanced organised social behaviour in mice fed with capsaicin diet. However, its mechanism of action has not been ascertained [33].

4.5 Learning and memory

The hidden-platform task of the Morris water maze is a test of visuospatial learning and memory in the mice and is also hippocampus dependent [39]. The use of extra-maze cues was employed in this task. On the other hand, visible-platform (cued) task of the Morris water maze is a non-hippocampal task and dependent on the dorsal striatum (caudate nucleus and putamen) of the basal ganglia [39]. The visible (cued) platform used a unique intramaze visual cue placed at the location of the escape platform.

The shorter the swim latency, the better the training process. Mice with learning disabilities or impairments were not able to quickly figure out the spatial location/position of the hidden platform, i.e., it took them a long time. Also, the steeper the gradient of swim latencies within the 3 day acquisition or reversal trainings, the better the learning curve, hence faster learning. Following the consumption of pepper and capsaicin diets, the swim latencies of the pepper and capsaicin groups were significantly longer than control in the first 3 days (acquisition training). This shows that pepper and capsaicin delayed learning process during the acquisition training [40].

During reversal training, the swim latencies of the test groups were also significantly longer on day 1 of the 3 day reversal training task, while on days 2 and 3, the values did not differ from control. This means that on days 2 and 3, the three groups learned equally while the control learned better on day 1. Visuospatial memory was also assessed during the probe trial (exploration without hidden platform). During this trial, it was expected that mice with good memory of the spatial location/position of the hidden platform would spend more time exploring the quadrant which had the platform during reversal training (North-East quadrant), but this was not observed in mice treated with pepper and capsaicin diets. They spent less time in the North-East quadrant. This means that they had memory impairment [40]. This is in contrast to the work by Kong et al. [41] which reported that capsaicin did not significantly alter the learning and memory performance in young adult mice but reduced the number of newly generated cells in the hippocampus. However, this is in line with the work by Kooshki et al. [42]. It is possible that the nociceptive effects of Capsaicin might have also affected learning and memory in the mice.

Learning and memory which are complex cognitive functions of the higher nervous centres encompass a variety of subcomponents with many interactions and overlaps [43]. Memories are stored in the brain by changing the basic sensitivity of synaptic transmission between neurons as a result of previous neural activity. The effects observed might have been due to the presence of the alkaloid called capsaicin. Since capsaicin is neurotoxic [9], it is likely that it impaired synaptic transmission between neurons by interfering with the basic sensitivity of the transmission in the hippocampus leading to impairment of learning and memory of the mice.

5. Conclusion

Consumption of both chilli pepper and capsaicin diets decreased pain perception, increased anxiety related behaviours, impaired learning and memory but enhanced social behaviour and motor coordination in mice. Therefore, it is likely that capsaicin, which is a powerful and stable alkaloid in chillies, may be one of the principles responsible for the observed neurobehavioural parameters in the experimental animals.

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