Journal of Advances in Medicine and Medical Research

24(7): 1-12, 2017; Article no.JAMMR.37032
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Long-term Consumption of *Capsicum annum* (Chili Pepper) and Capsaicin Diets Impairs Visuo-Spatial Learning and Memory in CD-1 Mice

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Authors' contributions

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

Article Information

DOI: 10.9734/JAMMR/2017/37032
Editor(s):
(1) Xin-an Liu, Neuroscience Department, the Scripps Research Institute, Scripps, Florida, USA.
Reviewers:
(1) Gerard G. Dumancas, Louisiana State University, USA.
(2) Dinithi Peiris, University of Sri Jayewardenepura, Sri Lanka.
(3) Weiting Wang, Tianjin Institute of Pharmaceutical Research, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/21975

Original Research Article

Received 27th September 2017
Accepted 13th November 2017
Published 18th November 2017

ABSTRACT

Background: *Capsaicin annum* (Chili pepper) is among the most consumed spices throughout the world. These spices/fruits contain chemicals called capsaicinoids. Capsaicin, the active principle in chilies is a major capsaicinoid responsible for up to 90% of the total pungency of pepper fruits. It is a generally known neurogenic, neurotoxic and analgesic agent. Since capsaicin is neurotoxic and has tendency to chemically interact with neurons, it may affect learning and memory.
Aim: It was therefore, the aim of this present study to investigate the effects of long-term consumption of capsaicin diet on learning and memory with a view of comparing them with those of chili pepper to see whether the effects of chili pepper on learning and memory can be attributed to capsaicin using adult CD-1 Swiss white mice as experimental animals.
Materials and Methods: Thirty male (30) 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. Feeding

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lasted for 28 days, during which there were daily measurements of food intake, water intake and body weight changes. Thereafter, their learning and memory abilities were assessed through their ability to locate the hidden platform model of Morris water maze apparatus.
Results: Pepper consumption reduced food intake but increased water intake in mice. The swim latencies of both capsaicin and pepper diet groups were significantly longer compared to control ($p<0.001$). The probe trial of the Morris water maze test showed a significantly shorter quadrant duration in the pepper and capsaicin groups compared to control ($p<0.001$ and $p<0.01$ respectively).
Conclusion: Long-term consumption of chili pepper and capsaicin diets impairs visuo-spatial learning and memory in mice.

Keywords: Capsicum annum; capsaicin; learning and memory.

1. INTRODUCTION

Chili pepper, perhaps the world's most prevalent spice consists of dried ripe fruit of *Capsicum annum,* belonging to the family *Solanaceae* [1, 2]. It is also known as chilies and amongst other species in the *Capsicum* genus, it is the most widely and extensively cultivated vegetable [3]. The other four main species in the *Capsicum* genus include *C. baccatum, C. chinense, C. frutescens and C. pubescens.* These peppers are widely used as spices in food industry and in a broad variety of medicinal applications worldwide [4]. 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 [4]. It is called *Ntokon in* Efik, *Ose 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 plant is not an annual crop and in the absence of winter frost can survive several seasons and grow into a large perennial shrub, [5]. The single flowers are an off-white (sometimes purplish) colour while the stem is densely branched and up to 60cm tall. The fruit is a berry and may be green, red, or yellow when ripe. While the species can tolerate most climates, *Capsicum annum* is especially productive in warm and dry climate.

The substance that gives chilies their hot sensation and intensity when ingested or contacted is the chemical compound, capsaicin and several other related chemicals collectively called capsaicinoids [6].

Capsaicin is an irritant to the skin, eyes and lungs [7]. Exposure to skin causes intense burning sensation while exposure to the eyes leads to intense tearing, conjunctivitis and blepharospasm. Oral ingestion of large amounts causes abdominal pain, vomiting and diarrhea.

Medically, capsaicin has been used as a topical analgesic agent in the preparations or formulations against arthritis [8]. Furthermore, since capsaicin causes neurogenic inflammation (burning and stinging sensations of hands, mouth and eyes), it is used in the formulation of defensive sprays. As reported by Kempaiah et al. [9], capsaicin demonstrates protective effects against cholesterol and obesity by speeding up metabolism through the release of stress hormones. Capsaicin decreases the activation of extracellular signal-regulated kinases (ERK) without markedly affecting p38 kinases and also reduces the number of newly generated cells in the dentate gyrus of the hippocampus [10]. Studies carried out by Nishiyama [11] showed that dietary ingestion of red bell pepper ameliorated the learning impairment in senescence accelerated mouse (SAMPS).

From the foregoing it can be gleaned that capsaicin is a highly irritant material requiring proper protective goggles, respirators and proper hazardous material handling procedures. It is an irritant to the skin, eye and lungs [12].

Since *Capsicum annum* (chili pepper) which forms a part of the diet of many people around the world and contains capsaicin which is neurotoxic [13], it is conceivable that it may affect neuronal activities in the body such as learning and memory. No previous studies conducted related to the effect of pepper (*Capsicum annum*) on learning and memory. Therefore, this present research investigated the comparative effects of long term consumption of capsaicin and chili

pepper on learning and memory, using mice as experimental models to check 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

Half-washed basin of fresh red chili pepper (*Capsicum annum*) was procured from Watt Market in Calabar, Nigeria. It was washed and sun-dried for 4 days. The dried samples were then pulverized 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. 40 g of the dry pepper was extracted with 100 mls of 98% absolute ethyl alcohol for 40mins in a continuous extraction apparatus (Soxhlet extractor). About 100mls 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 mls of normal saline (with each ml containing 50 mg of capsaicin) to form a stock solution for the toxicity study.

2.2 Preliminary Experiments

2.2.1 Determination of lethal dose of capsaicin and chili pepper

Lethality study was done using Karber's method [14]. Sixty (60) mice used for the lethal study were divided into 2 sets. Each set had 6 groups of 5 mice each. Set 1 was for chili pepper lethality study while set 2 was for capsaicin lethality study. In the chili pepper set, the control group was given 1ml of normal saline, while the remaining 5 groups were administered 800 mg/kg, 1000 mg/kg, 1200 mg/kg, 1400 mg/kg, and 1600 mg/kg in 1ml of normal saline respectively. In the capsaicin set, the control group was administered 1 ml of normal saline, whereas the remaining 5 groups received 25 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg in 1 ml of normal saline respectively. All administrations were orally done. The number of mortality in each group after 24 hours was recorded. The LD_{50} was then calculated using probit kill of the doses.

To determine the safe and effective dose to use, graded percentages of capsaicin diet (4% to 20%) and chili pepper diet (5% to 40%) were given to the mice.

2.2.2 Preliminary phytochemical analysis of chili pepper

Both the ethanol and aqueous extracts obtained were subjected to various chemical tests to detect the chemical constituents present in them using standard methods. For qualitative analysis, Salkowski's test was used for glycosides, Frothing's test for Saponins, Fehling's test for reducing compounds, Wagner's test for Alkaloids. Color change tests for Flavonoids, Polyphenols and Tannins. For quantitative analysis, gavimetric, spectrophotometric and Benedict's quantitative test methods were used [15,16,17,18].

2.2.3 Estimation of the capsaicinoids in chili pepper (*Capsicum annum*)

The extraction of the capsaicinoids was performed according to the modified method described by Thomas et al. [19]. 25 g of the pulverized sample was extracted with 50 mls of acetone. The sample was further homogenized using a laboratory homogenizer for 5 min until all the tissue was macerated in 25 mls of acetone. The organic extracts were centrifuged and solvents evaporated under reduced pressure at 40℃ for the collection of the precipitate. The crude extract was dissolved in 2.5mls of water and then subjected to column chromatography (CC) on silica gel (ZCX-type 2) and subsequently eluted with an isocratic solvent system of petroleum ether, ethyl acetate and methanol (75:20:5). Five fractions were collected (F1-F5). All fractions were applied on TLC plate coated with silica gel (60 GF254) and developed in the same solvent system as in column chromatography.

Two plates were used. One of them was sampled with the five fractions while the other one was sampled with the capsaicin standard solution (2 mg/ml). In order to identify the capsaicinoids, the plates were sprayed with the solution of 2,6-dichlorochinonechloimide. The fractions containing capsaicinoids were mixed, evaporated under vacuum until dryness at 40℃

and dissolved in 2 mls of methanol for Gas Chromatography analysis.

2.3 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 monitored. They were randomly assigned into three groups, namely; control group that received normal rodent chow, pepper group that were fed 20% chili pepper diet and capsaicin group that were given 10% capsaicin diet. Each group comprised 10 mice. Each mouse was allowed drinking water *ad libitum*. After every 24 hours, the amount of food and water left was subtracted from the initial amount given to obtain daily food and water intake per mouse. This treatment was done for 28 days and within this period, their beddings, feed and water were hygienically handled and changed daily. Body weights of the animals were also taken every 3 days. Thereafter, the animals were assessed for their learning and memory capabilities.

2.4 Assessment of Learning and Memory

The Morris water maze developed by Richard Morris [20] for assessing visuo-spatial 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 eight days. The first three days were for acquisition training with an invisible platform. The next three 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 the60 seconds. 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 analyzed by one way analysis of variance (ANOVA)

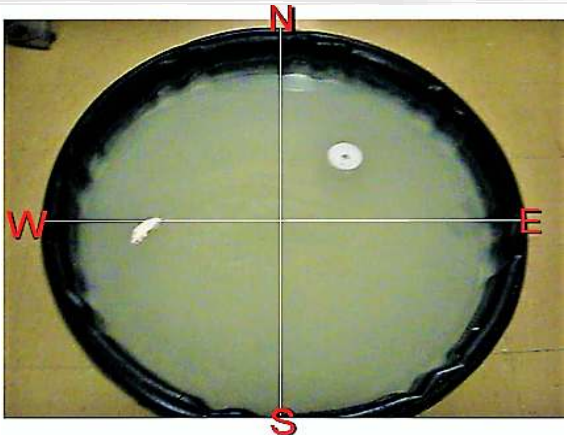


Plate 1. Mice exploring the quadrants of the Morris water maze

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 mean) and *p* value less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Phytochemical Screening of Pepper

Qualitatively, the ethanol extract shows that polyphenol was present in excess, reducing compounds and glycosides were present in moderate amounts. Alkaloids, tannins and flavonoids were present in scanty amount. In the aqueous extract, saponins, reducing compounds and polyphenols were present in excess amount. Alkaloids and flavonoids were present in moderate amount, while glycosides and tannins were scantily present. Saponin was absent in the ethanol extract while phlobatannins, anthraquinones and hydroxymethyl anthraquinones were absent in both the aqueous and ethanol extracts (Table 1).

Quantitatively, dry matter of pepper extract contained more of saponins (4.00 ± 0.1) mg/ml, followed by reducing compounds (2.86 ± 0.01) mg/ml, polyphenols (2.80 ± 0.1) mg/ml, flavonoids (1.20 ± 0.1) mg/ml, glycoside (1.07 ± 0.01) mg/ml, alkaloids (0.60 ± 0.1) mg/ml and tannins (0.09 ± 0.1) mg/mls (Table 2).

3.2 Capsaicinoids Extraction/Analysis Results Using Current / Gas Chromatography

Table 3 shows the various substances found in chili pepper as analyzed by high power liquid chromatography (HPLC). The analysis shows the presence of nordihydrocapsaicin (4.48565 mg/100 g), capsaicin (109.88724 mg/100 g), dihydrocapsaicin (25.27855 mg/100 g), homocapsaicin (6.37513 mg/100 g) and

Table 1. Results of phytochemical screening of chili pepper

Chemical constituents		Ethanol extract	Aqueous extract
1.	Alkaloids	+	++
2.	Glycoside	++	+
3.	Saponins	-	+++
4.	Tannins	+	+
5.	Flavonoids	+	++
6.	Reducing compounds	++	+++
7.	Polyphenol	+++	+++
8.	Phlobatannins	-	-
9.	Anthraquinone	-	-
10.	Hydroxymethyl Anthraquinones	-	-

Keys: +++ = present in excess; ++ = present in moderate amount
+ = present in scanty amount; - = absent

Table 2. Quantitative estimation of the phytochemicals in chili pepper

Name of sample	Alkaloids (mg/ml)	Glycosides (mg/ml)	Saponins (mg/ml)	Flavonoids (mg/ml)	Polyphenos (mg/ml)	Reducing compounds (mg/ml)	Tannins (mg/ml)
Pepper	0.60±0.1	1.07±0.01	4.00±0.1	1.20±0.1	2.80±0. 1	2.86±0.01	0.09±0.1

Values are mean ± sd

Table 3. Analysis of capsaicinoids in pepper (*Capsicum annum*) by High performance liquid chromatography (per 100 g)

S/N	Capsaicinoids	Area (PA)	Amount/area	Amount (mg/100 g)
1	Nordihydrocapsaicin	40.79727	1.83250e-2	4.48565
2	Capsaicin	229.92805	7.96533e-2	109.88724
3	Dihydrocapsaicin	240.04170	1.75515e-2	25.27855
4	Homocapsaicin	129.37523	8.21272e-4	6.37513e-1
5	Homodihydrocapsaicin	278.73935	5.01968e-4	8.39509e-1
	Total	918.88160		141.12845

homodihydrocapsaicin (8.39509 mg/100 g). The total amount of capsaicinoids found was 141.12845 mg/100 g at an area of 918.88160PA. Calibration curves were obtained for all the concentrations of capsaicinoids.

3.3 Lethality Study of Capsaicin and Pepper

The lethal dose of capsaicin following graded doses of 25 to 400 mg/kg was 34.07 mg/kg (Fig. 1). For chili pepper, the LD_{50} was 932.44mg/kg after graded doses of 800 to 1600mg/kg were tested (Fig. 2).

3.4 Comparison of Food Intake of the Different Experimental Groups

From days 1-8, the daily food intake in the pepper group was lower than that of control and capsaicin groups ($p< 0.001$). Thereafter, it increased and remained steady above the capsaicin group but below the control on days 20 and 28. Food intake in the capsaicin group was significantly lower than control from days 4 to 28 ($p< 0.05 – 0.001$) (Fig. 3A). Over the 28 day period, the mean food intake in the capsaicin diet-fed group was significantly lower ($p<0.05$) when compared to control but showed no significant difference with the pepper group (Fig. 3B).

3.5 Comparison of Water Intake of the Different Experimental Groups

Capsaicin and pepper diet-fed mice drank more water than the control mice within the first 12 days ($p< 0.05- 0.001$). Thereafter, only pepper diet-fed mice continued to drink significantly more water than the control and capsaicin diet-fed mice (Fig. 4A). Over the 28 day period, the mean water intake of both the pepper diet-fed and capsaicin diet-fed mice was significantly higher compared to control ($p<0.01$ and $p<0.05$ respectively) (Fig. 4B). No difference was observed between the two test groups.

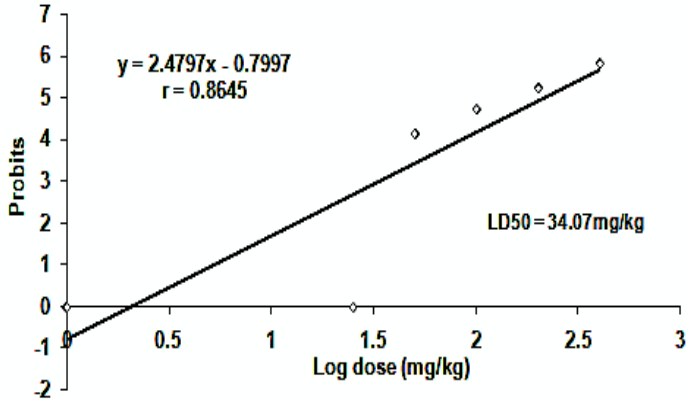


Fig. 1. Lethality study for the determination of LD_{50} of capsaicin in mice

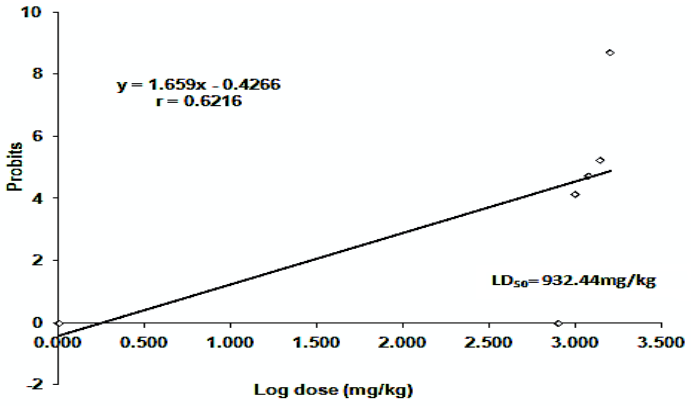
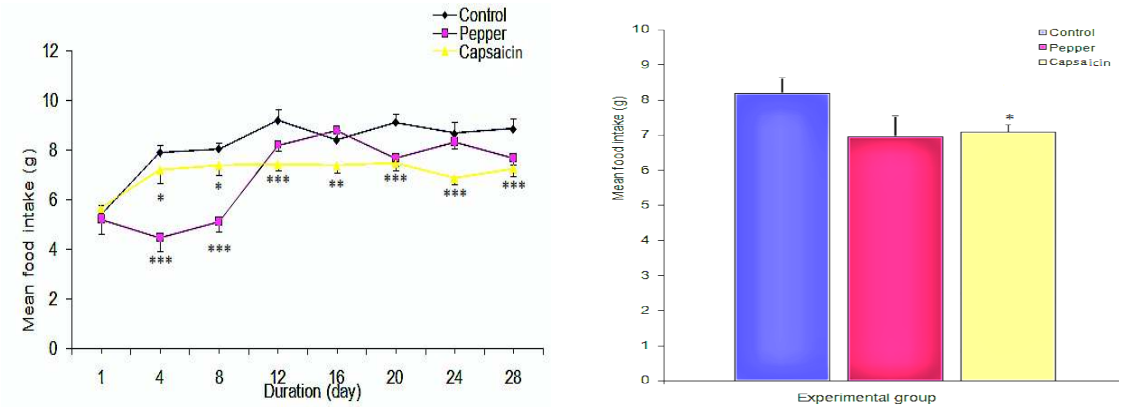


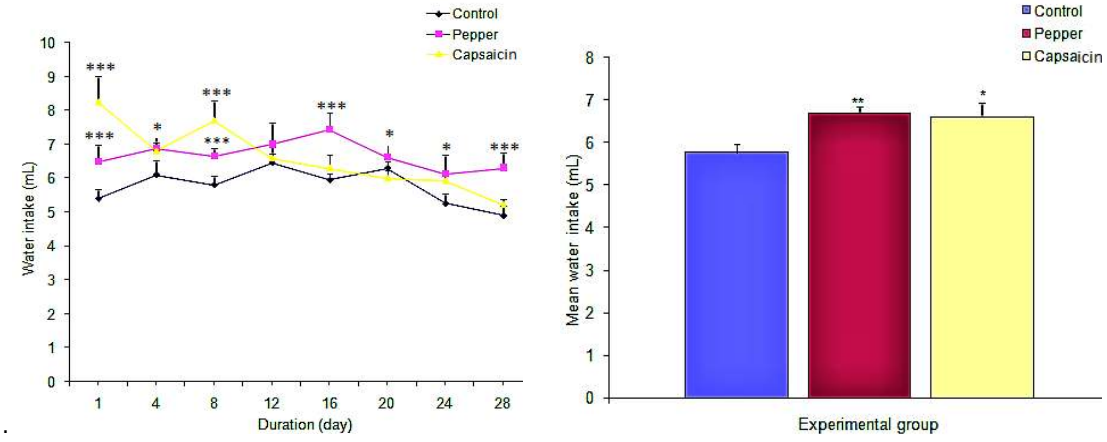
Fig. 2. Lethality study for determination of LD_{50} of pepper extract in mice



(A) **(B)**

Fig. 3. Comparison of the (A) daily food intake and (B) mean food intake of the different experimental groups

*Values are expressed as mean ± SEM, n=10; * =p<0.05, ** =p< 0.01, *** =p<0.001 vs control*



(A) **(B)**

Fig. 4. Comparison of the (A) daily water intake and (B) mean water intake of the different experimental groups

*Values are expressed as mean ± SEM, n=10; *=p<0.05, ** =p< 0.01, *** =p<0.001 vs control*

3.6 Comparison of Body Weight Changes of the Different Experimental Groups

The body weight changes for the pepper and capsaicin groups were significantly lower (p<0.001) compared to the control. However, the body weight of the capsaicin group was lower than that of the pepper group (Fig. 5).

3.7 Comparison of Swim Latency in the Morris Water Maze Test for Learning and Memory

During the acquisition training, the capsaicin group had a significant longer (p<0.001) swim latency on days 2 and 3 compared to control (Fig. 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 (Fig. 6B).

3.8 Comparison of Quadrant Duration in the Morris Water Maze Test for Learning and 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) (Fig. 7).

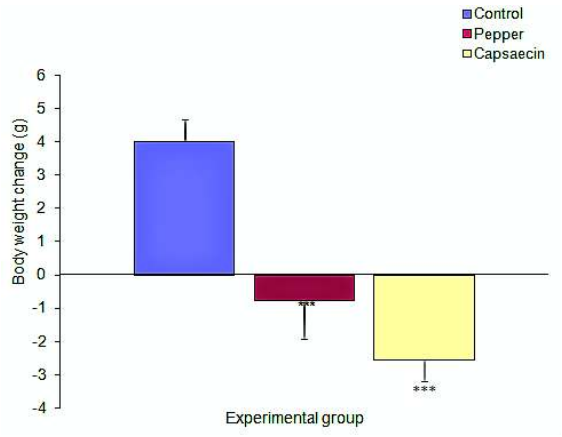


Fig. 5. Comparison of the body weight changes of the different experimental groups

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

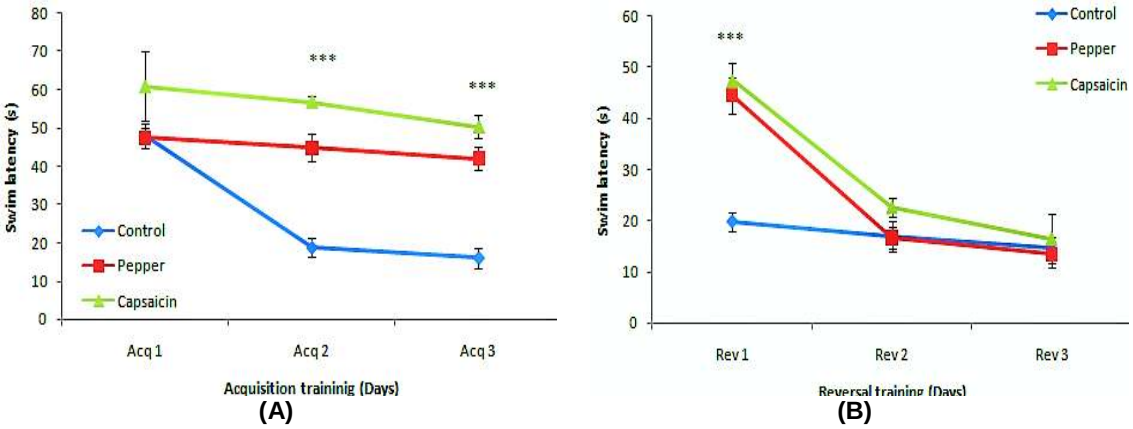


Fig. 6. Comparison of swim latency in Morris water mazetest during (A) Acquisition training and (B) Reversal training of the different experimental groups

*Values are expressed as mean ± SEM, n =10; *** =p<0.001 vs control*

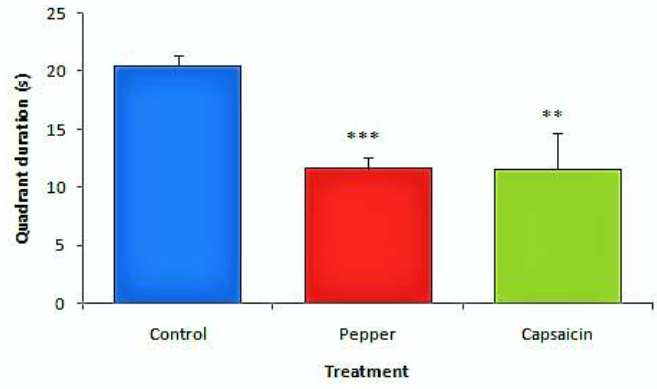


Fig. 7. Comparison of quadrant duration in Morris water maze test of the different experimental groups

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

3.9 Comparison of Swim Latency during the Visible Platform Task of Morris Water Maze Test

The results show no significant difference between the pepper and capsaicin diet groups when compared to control (Fig. 8).

4. DISCUSSION

Phytochemical analysis of ethanol extract of chili pepper showed the presence of polyphenols in excess, reducing compounds and glycosides in moderate amounts, while alkaloids, tannins and flavonoids were present in scanty amounts. Saponins, reducing compounds and polyphenols were found in excess in the aqueous extract. Alkaloids and flavonoids were in moderate amounts, while glycoside and tannins were scantily present. The quantitative screening result shows that saponins, reducing compounds and polyphenols were the most abundant substances in pepper. The lethality results obtained showed that capsaicin is more lethal than pepper.

Over the 28 day feeding period, the mean food intake of the capsaicin diet-fed group was lower than the control. The food intake in the pepper diet-fed group appeared lower than control but was not significant. On the other hand, the water intake of the test groups was significantly higher than control over the twenty-eight day period. Food intake is controlled by feeding centers in the hypothalamus. When the hunger center (situated in the lateral hypothalamus) is stimulated, an animal searches for food to eat and only stops when the ventral medial hypothalamus is stimulated or when there is inhibition of the hunger center [21,22]. It is likely that pepper inhibited the hunger center, resulting in reduced intake of food in mice and this invariably led to the reduction in the body weights of the mice. It is also possible that the test diets were not palatable to the mice and caused increase in water intake by stimulating the hypothalamic thirst center leading to increased water consumption by the mice. There is no evidence showing that weight loss is directly correlated with ingesting capsaicin, but there is a positive correlation between ingesting capsaicin and a decrease in weight regain. The effects of capsaicin are said to cause "a shift in substrate oxidation from carbohydrate to fat oxidation" [23]. This leads to a decrease in appetite as well as a decrease in food intake [23]. Short-term studies suggest that capsaicin aids in the decrease of weight regain. However, long-term studies are limited because of the pungency of capsaicin [24] Another recent study has suggested that the ingestion of capsaicinoids can increase energy expenditure and fat oxidation through the activation of brown adipose tissue (BAT) in humans from the effects of the capsaicin and thus decrease weight gain [25].

The hidden-platform task of the Morris water maze was a test of visuo-spatial learning and memory in the mice and was also hippocampus dependent [26]. The use of extra-maze cues was employed in this task. On the other hand, visible-platform (cued) task of the Morris water maze was a non- hippocampal task and dependent on the dorsal striatum (caudate nucleus and putamen) of the basal ganglia [26].

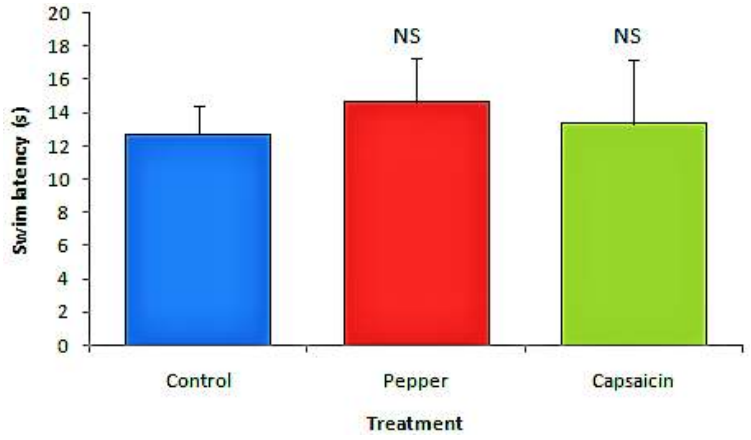


Fig. 8. Comparison of swim latency during the visible platform task in Morris Water Maze test of the different experimental groups.

Values are expressed as mean ± SEM, n =10; No significant difference between the groups

The visible (cued) platform used a unique intra-maze 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 three 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 three days (acquisition training). This shows that pepper and capsaicin delayed learning process during the acquisition training. During reversal training, the swim latencies of the test groups were also significantly longer on day 1 of the three 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.

The cued version of Morris water maze assesses cued learning and visual integrity of the animals tested. In this cueing procedure, the escape platform protrudes above the water surface. Shorter swim latency indicates improved cued learning. From the results, the swim latencies in cued learning were not significantly different compared to control. This means that both the test groups and control learned equally.

Visuo-spatial 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. This is in contrast to the work by Hong et al. [8] 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 hypocampus. However, this is in line with the work by Kooshki et al. [27]. 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 centers encompass a variety of subcomponents with many interactions and overlaps [28]. 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

Long-term consumption of capsaicin or chili pepper containing diet impairs learning and memory. Therefore, capsaicin which is a powerful and stable alkaloid in chilies may be one of the constituents responsible for the impairment of learning and memory in mice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors herein declare that the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) as well as national laws on care of animals were strictly adhered to during the experiments. Appropriate approval was also obtained from the local ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yoon JY, Green SK, Tshanz AJ, Tsou SCS, Chenge LC. Pepper improvement for the tropics, problems and approach. In: Tomato and Pepper Production in the Tropics. Asian Vegetable Research and Development Center, AVRDC Shantung Taiwan. 1989;86-90.
2. Nwokem CO, Agbaji EB, Kagbu JA, Ekanem EJ. Determination of capsaicin content and pendency level of five different peppers grown in Nigeria. New York Sci. J. 2010;3(9):17-21.

3. Estrada B, Bernal MA, Diaz J, Pomar F, Merino F. Capsaicinoids in vegetative organs of *Capsicum annum* L. in relation to fruiting. Journal of Agricultural and Food Chemistry. 2002;50:1188-1191.
4. Zimmer AR, Leonardi B, Zimmer ER, Kalinine E, de Souza DO, Portela LV, Gosmann G. Long-term oral administration of *Capsicum baccatum* extracts does not alter behavioral, hematological and metabolic parameters in CF1 Mice. Evid Based Complement Alternat Med. 2012; 2012:196358.
DOI: 10.1155/2012/196358
5. Katzer G. Paprika (*Capsicum annum* L.). Published May 27, 2008.
(Accessed on October 20, 2017)
Available:htpps://www.uni-graz.at/~katzer/engl/caps_ann.html
6. Perucka I, Materska M. Phenylalanine Ammonia-lyase and antioxidant activities of lipophilic fraction of fresh pepper fruits *Capsicum annum* L. Innov. Food SciEmerg. Techno. 2001;2:189-192.
7. Johnson W. Final report on the safety assessment of *Capsicum annum* extract, *Capsicum annum* fruit extract, *Capsicum annum* resin, *Capsicum annum* fruit powder, *Capsicum frutescens* fruit, *Capsicum frutescens* fruit extract, *Capsicum frutescens* resin and capsaicin. Int. J. Toxicol Suppl. 2007;1:3-106.
8. Deal CL, Schnitzer TJ, Lipstein E, Seibold JR, Stevens RM, Levy MD, Albert D, Renold F. Treatment of arthritis with tropical capsaicin: A double blind-trial. Clinical Therapy. 1991;13(3):383-395.
9. Kempaiah RK, Manjunatha H, Srinivasan K. Protective effect of dietary capsaicin on induced oxidation of low-density lipoprotein in rats. Journal of Molecular and Cellular Biochemistry. 2005;275:7-13.
10. Kong KH, Kim HK, Song KS, Woo YS, Choi WS, Park HR, Park M, Kim ME, Kim MS, Ryu JS, Kim HS, Lee J. Capsaicin impairs proliferation of neural progenitor cells and hippocampal neurogenesis in young mice. J Toxicol Environ Health A. 2010;73(21-22):1490-501.
11. Nishiyama T. Interaction between intrathecal morphine and glutamate receptor antagonists in formalin test. Eur J Pharmacol. 2000;395:203–210.
12. Johnson Wilbur. "Final report on the safety assessment of *Capsicum annuum* extract, *Capsicum annuum* fruit extract, *capsicum Annuum* resin, *Capsicum annuum* fruit powder, *Capsicum frutescens* fruit, *Capsicum frutescens* fruit extract, capsicum frutescens resin, and capsaicin. Int. J. Toxicol. 2007;26(Suppl 1):3–106.
13. Scherrer G, Befort K, Contet C, Becker J, Matifas A, Kieffer BL. The delta agonists DPDPE and deltorphin II recruit predominantly mu receptors to produce thermal analgesia: A parallel study of mu, delta and combinatorial opioid receptor knockout mice. Eur J Neurosci. 2004; 19(8):2239-2248.
14. Turner R. Acute toxicity: The determination of LD_{50}. In: Screening Methods in Pharmacology. New York: Academic Press. 1965;61-63.
15. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd.: London. 1973;279.
16. Trease GE, Evans MC. Textbook of pharmacognosy. London Bailliare Tindall. 1983;12:193.
17. Giulia DC, Nicola M, Angelo AI, Francesco C. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sci. 1999;65(4):337-353.
18. Villaño D, Fernández-Pachon MS, Moyá ML, Troncoso AM, Garcia-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 2007;71(1):230-235.
19. Thomas BV, Schreiber AA, Weisskopf. Simple method for quantification of capsaicinoids in pepper using capillary gas chromatography. J Agric. Food chem. 1998;46(7):2655-2663.
DOI: 10.1021/jf970695w
20. Morris R. Developments of a water maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods. 1984;11:47-60.
21. Ganong WF. Review of medical physiology, 21st ed. McGraw Hill Baston. George Ltd. 2005;239.
22. Nmaju AU, Bisong SA, Nwankwo AA, Joshua IE, Osim EE. Comparative effects of *Garcinia kola* and coffee diets on learning and memory in mice. British Journal of Medicine and Medical Research. 2014;4(2):731-746.
23. Lejeune MP, Kovacs EM, Westerterp-Plantenga MS. Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. Br J Nutr. 2003; 90(3):651-659.

24. Diepvens K, Westerterp KR, Westerterp-Plantenga MS. Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. Am J Physiol Regul, Integr Comp Physiol. 2006; 292(1):R77-85.
25. Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. Nonpungent capsaicin analogs (Capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. Am J Clin Nutri. 2012; 95(4):845-850.
26. McDonald RJ, White NM. Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. Behavioural Neural Biology. 1994;61:260-270.
27. Kooshki R, Abbasnejad M, Esimaeili-Mahani S, Raoof M. The role of trigeminal nucleus caudalis orexin 1 receptors in orofacial pain transmission and in orofacial pain-induced learning and memory impairment in rats. Physiol Behav. 2016; 157:20-27.
28. Brem A, Ran K, Pascual-Leone A. Learning and memory. Handb Clint Neurol. 2013;116:693-737.

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