Journal of Advanced Zoology

ISSN: 0253-7214
Volume ***45*** *Issue* ***2 Year 2024*** *Page* ***1261:1281***

Role Of TRPV I Receptor In Bilateral Common Carotid Artery Occlusion (BCCAO) Induced Vascular Dementia In Rat.

Supriya Mishra[1]*, (Prof.) Dr. Lubhan Singh[2]

[1]Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut-250005, UP, India".Email Id: supriya.initative@gmail.com, contact No.+9188264811091*
**"SRM Modinagar College of Pharmacy, SRM Institute of Science andTechnology, NCR Campus, Delhi-Meerut Road, Ghaziabad-201204, UP, India".*
[2]Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut-250005, UP, India".

****Corresponding Author:** Supriya Mishra*
** *"SRM Modinagar College of Pharmacy, SRM Institute of Science andTechnology, NCR Campus, Delhi-Meerut Road, Ghaziabad-201204, UP, India".*

Article History

Received: 02/01/2024
Revised: 20/01/2024
Accepted: 02/02/2024

Abstract

Dementia is a devastating disorder that commonly affects people over the age of 65. Alzheimer's disease and vascular dementia are the most common forms of dementias, A number of studies have implicated cardiovascular risks as important factors in the development of dementia. Loss of cognitive function and vascular risk factor produce pathological changes into the brain. Changes are Infarcts, Ischemia hypoperfusion, Hemoragic Brain and Atrophy (Degeneration of cells), Neurofibrillary tangles impairment. Bilateral Common Carotid Artery occlusion (BCCAO) has been considered as a critical cause for the development of cognitive decline and dementia of vascular origin. In our study hypoperfusion reduced oxidative stress, nitric oxide level, Acetylcholine, SOD (Superoxide Dismutase). TRPV1 Receptors have been reported to be beneficial in improving memory deterioration.

Aim of this study is to explore the role of capsaicin in Bilateral Common Carotid Artery occlusion (BCCAO) induced vascular

CC License
CC-BY-NC-SA 4.0

dementia .In clinical research capsaicin role is of antioxidant and other one shows anti-inflammatory actions. It showed significant cognitive deficits, cholinergic dysfunction (increased acetyl cholinesterase-AChE) activity along with increased brain oxidative stress (brain thiobarbituric acid reactive species), glutathione, as well as superoxide dismutase with an increase in malondialdehyde levels) and TTC (2,3,5 Triphenyltetrazolium chloride staining).

Furthermore, treatment of capsaicin reduced BCCAO induced learning and memory deficits.(Morris water maze-MWM), locomotion (Actophotometer), and limited cholinergic dysfunction, oxidative stress, and tissue damage, Estimation of Brain Total Protein, infarct volume suggesting that capsaicin TRPV1 Receptors may provide benefits in BCCAO induced VaD.

Keywords – Antioxidants, Neurofibrillary tangles, hypoperfusion, 2, 3,5 Triphenyltetrazolium Chloride

1. INTRODUCTION

Neurodegenerative disorders are one of the most frequent causes of death and disability worldwide and have a significant clinical and socio-economic impact (Cowenand Kandel 2001). Neurodegenerative disorders are characterized by progressive and irreversible loss of neurons from specific regions of the brain (Prakash et al 2012).

Dementia disease is a devastating neurodegenerative disorder manifested by deterioration in memory and cognition, impairment in performing activities of daily living, and many behavioral and neuropsychiatric illnesses (Zhang 2012).

VaD is the second most common kind of dementia and accounts for about 20-30 percent of all common cases. In VaD loss of cognitive function and vascular risk factors produce pathological changes into the brain (Zhao *et al.,* 2014).

Vascular dementia is caused by decreased or interrupted blood flow to parts of the brain, which is predominantly caused by cerebrovascular accident or stroke (Watari, and Gatz, 2002).

Research indicates there is a common pathology between Alzheimer's disease and vascular dementia Risk factors such as hypertension, Type 2 diabetes, and high cholesterol increases the risk of vascular dementia (Sadowski *et al.,* 2004).

TRPV-1 RECEPTOR

The transient receptor potential vanilloid type 1 (TRPV1) is a nonselective cation channel that consists of six transmembrane domains.

capsaicin has a number of positive health impacts on humans, including antioxidant activity, anti-inflammatory, anti-mutagenic, anti-metastatic, and anti-depressant qualities. Additionally, capsaicin shows neuroprotective properties against a variety of neuropathological and neurological diseases. Previous research has indicated that vanillin's medicinal qualities, such as its anti-inflammatory, antioxidant, and anti-cancer capabilities, may have a range of positive effects on brain damage.

2.0 MATERIALS AND METHODS

2.1 DRUGS AND CHEMICALS

All the drug solutions and suspensions were freshly prepared before use. Vanillin and capsaicin was obtained from (Mankind Pharma Ltd., India). Sodium Hydroxide Solution, Thiobarbituric acid, sodium-potassium tartrate, sodium carbonate, Copper Sulphate ($CuSO_4$) Solution, sodium dodecyl sulphate, n-butanol, pyridine, 1, 1, 3, 3-tetra methoxy propane, glutathione (GSH), trichloroacetic acid, disodium hydrogen phosphate, DTNB [5, 5`-dithiobis (2-nitrobenzoic acid), sodium citrate, acetylthiocholine chloride (Gupta & Sharma 2014).

2.2 ANIMALS SELECTION

Wistar albino rats (3-5 months aged), weighing 200–250 g were used and were kept in animal house with water and standard laboratory pellet chow diet under standard laboratory condition (26+ 1^{o} C) *ad libitum*. The animals were housed in standard poly carbonate cages, and maintained on 12 h light and 12 h dark cycle. The experiments were performed between 9.00 and 18.00 h in a semi-sound-proof laboratory. The animals were acclimatized to the laboratory environment seven days before the behavioral study and were kept in the laboratory till the study ends. Protocol used in this study was properly sanctioned by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (**Reg.No.1204/PO/Re/S/08/CPCSEA/22/04).**

2.3 SELECTION OF DOSE

On the basis of previous reported literature (Ludy et al., 2012)

2.4 SURGICAL PROCEDURE

We have followed permanent bilateral common carotid arteries ligation method BCCAO rat model is a model of acute/subacute brain ischemia more than a model of chronic hypoperfusion of the brain. Permanent bilateral common carotid arteries ligation was done (Azzubaidi1 *et al*., 2012). Permanent carotid ligation in rats model of cerebral hypoperfusion has been increasingly used as a paradigm for neurodegenerative disorders with permanent bilateral ligation of common carotid arteries (Farkas *et al*., 2007) creating a state of oligemia (cerebral hypoperfusion) that in the long run leads to neurodegeneration predominantly to pyramidal hippocampal neurons in charge of spatial (place) learning and memory (Nakazawa *et al*., 2004). Thus, BCCAO may be utilized to cause a permanent and persistent cerebral hypoperfusion. Briefly, rats were anesthetized with ketamine (anesthetic agent) in a dose of 100 mg/ kg (Tsukamoto *et al*., 2014). Both carotid artery was identified and carefully separated from the vagus nerve. After that the carotid artery was doubly ligated with silk suture (4–0) just below the bifurcation into internal and external carotid arteries. After surgery, rats were placed under a heating lamp for the prevention of hypothermia until full recovery from general anesthesia. All surgical procedures were performed under aseptic conditions. Sham-operated animals underwent the same surgical procedure without carotid arteries ligation (Azzubaidi1 *et al*., 2012) (Gupta *et al*., 2014a).

2.5 EXPERIMENTAL PROTOCOL

In the present study, total six groups were employed and each group was consisted of five wistar albino Rats.

GROUP I - SHAM CONTROL

Sham surgery was performed on the animals without ligating both carotid arteries for 10-15 minutes. After that the animals were sutured back. Animals were exposed to MWM, 26th day onwards. Acquisition trials were performed from 26th to 29th day and retrieval trials were performed on the 30th day on MWM.

GROUP II – BCCAO+ VEHICLE CONTROL GROUP (CMC)

BCCAO were performed and animals were administered 0.5% w/v sodium carboxy-methylcellulose i.e. CMC (10 ml/ kg orally. once daily) for 26 days followed by exposure to MWM. Acquisition trials were performed from 26th to 29th day and retrieval trials were performed on 30 th day on MWM.

GROUP III - DRUG-I PER SE (CAPSAICIN)

Animals were administered capsaicin (10 mg/ kg orally, once daily) for 26 days followed by exposure to MWM. Rest of the procedure was the same as described in group II.

GROUP IV - BCCAO + VANILLIN

A permanent bilateral occlusion of the common carotid arteries (BCCAO) with drug occlusion was performed on the animals. Animals were exposed to MWM, 26th day onwards. Acquisition trials were performed from 26th to 29th day and retrieval trials were performed on the 30th day on MWM (Gupta & Sharma, 2014).

GROUP V- BCCAO+DRUG DOSE-I (CAPCASIN)

A permanent bilateral occlusion of the common carotid arteries occlusion was performed on the animals. Animals were administered with low dose of (10 mg/ kg orally, once daily) for 26 days Rest of the procedure was the same as described in group IV (Singh & Sharma, 2016).

GROUP VI- BCCAO+DRUG DOSE-II (CAPCASIN)

BCCAO animals will be administered with DRUG-I (High Dose of 30 mg/ kg orally once daily) for 30 days and Rest of the procedure was the same as described in group V (Singh & Sharma, 2016).

2.6 BEHAVIOURAL PARAMETERS

2.6.1 ASSESSMENT OF LEARNING AND MEMORY USING MORRIS WATER MAZE

Morris Water Maze (MWM) test was employed to assess learning and memory of rats (Morris, 1984; Sain *et al.*, 2011). The MWM procedure was based on a principle, where the animals were placed in a large pool of water, as animals dislike swimming, their tendency to escape from the water being accomplished by finding an escape platform. MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28±1˚C). The water was made opaque with nontoxic white colored dye. The tank was divided into four equal quadrants with a help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm^2), painted in white was placed 1 cm below surface of water inside the target quadrant. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials on each day with a gap of 5 min. The rat was gently placed in the water of the pool between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 sec to locate submerged platform. Then, it was allowed to stay on the platform for another 20 sec. If it failed to find the platform within 120 sec, it was guided gently onto the platform and allowed to remain there for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or learning. Animal was subjected to

four acquisition trials daily for four consecutive days. On fifth day, the platform was removed and each rat was allowed to explore in the pool for 120 sec. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrant searching for the hidden platform was noted as an index of retrieval (memory) (Gupta *et al*., 2015).

2.6.2 ACQUISITION TRIAL

Each rat was subjected to four trials on each day. A rest period of 5 min was allowed in between each trial. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four acquisition trials was changed as described below and Q4 was maintained as target quadrant in all acquisition trials.

Day1	Q1	Q2	Q3	Q4
Day2	Q2	Q3	Q4	Q1
Day3	Q3	Q4	Q1	Q2
Day4	Q4	Q1	Q2	Q3

Mean escape latency time (ELT) calculated for each day during acquisition trials day 4 ELT was used as an index of acquisition.

2.6.3 RETRIEVAL TRIAL

On fifth day the platform was removed. Rats were placed in water maze and allowed to explore for 120 sec. Each rat was subjected to four such trials and each trial was started from different quadrant. Mean time spent in all three quadrants i.e. Q1, Q2 and Q3 were recorded and the time spent in the target quadrant i.e. Q4 in search of missing platform provided an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study. All the trials were completed between 09.00 to 18.00 hrs in a semi sound proof laboratory.

2.7 LOCOMOTOR ACTIVITY

2.7.1 ACTOPHOTOMETER

The locomotor activity (horizontal activity) can be simply measured using an actophotometer that operates on photoelectrical cells that are connected in circuit with a counter. Once the beam of light falling on the photo cell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves, rats may be used for testing in this equipment (Wells *et al*., 2009).

2.7.2 PROCEDURE

Weigh the animals (200-250g rats) and range them. Turn on the equipment (check & ensurethat all photo cells are working the photo cells are working for accurate recording) and placed on an individual basis each rats in the activity cage for 10 minutes. Note the basal activity score of all the animals. Drug is injected and after30mins re-test every rats for activity scores for 10 minutes. Note the difference in the activity, before & after drug administration. Calculate percent decrease in motor activity.

2.8 BIOCHEMICAL ESTIMATIONS

2.8.1 DISSECTION AND HOMOGENIZATION

After the assessment of behavioral parameters, animals were sacrificed by decapitation. Brain of each animal was removed by putting on ice and weighed individually. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g at 4°C for 15 min. An aliquot of supernatant was separated and used for biochemical estimations (Gupta & Sharma 2014).

2.8.2 ESTIMATION OF BRAIN TOTAL PROTEIN

The brain total protein was determined by Lowry's method with slight modification (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard. 0.15 ml of supernatant of tissue homogenate was diluted to 1 ml than 5 ml of Lowry's reagent was added. The contents were mixed thoroughly and the mixture was allowed to stand for 15 min at room temperature. Then 0.5 ml of Folin-Ciocalteu reagent was added and the contents were vortexed vigorously and incubated at room temperature for 30 min. The standard curve was plotted using 0.2-2.4 mg/ml of BSA. The protein content was determined spectrophotometrically (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 750 nm. Protein concentration was expressed as mg/ml of supernatant.

2.8.3 ESTIMATION OF THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in brain was performed according to the method of Ohkawa *et al.*,(Okhawa *et al.*,1979). 0.2 ml of supernatant of homogenate was pipetted out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 1 h at 95 ^{0}C, then cooled and added 1 ml of distilled water followed by addition of 5 ml of n-butanol-pyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 g for 10 min. The absorbance of developed pink color was measured spectrophotometrically (DU 640B

spectrophotometer, Beckman Coulter Inc., CA, USA) at 532 nm. A standard calibration curve was prepared using 1-10 nM of 1, 1, 3, 3-tetra methoxy propane. The TBARS value was expressed as nanomoles per mg of protein.

2.8.4 ESTIMATION OF REDUCED GLUTATHIONE (GSH)

The reduced glutathione (GSH) content in tissue was estimated using method of Beutler *et al.*,(Beutler *et al.*,1963). The supernatant of homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 1000 g for 10 min at 4 ^{0}C. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Then 0.25 ml of 0.001 M freshly prepared DTNB [5, 5`-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added and absorbance was noted spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 412 nm. A standard curve was plotted using 10-100 µM of reduced form of glutathione and results were expressed as micromoles of reduced glutathione per mg of protein.

2.9 ASSESSMENT OF BRAIN ACETYLCHOLINESTERASE (ACHE) ACTIVITY

The whole brain AChE activity was measured spectrophotometerically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 420 nm by the method of Ellman et al. with slightmodification (Ellman et al., 1961; Koladiya et al., 2008, 2009; Sain et al., 2011). Briefly, this was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions.

2.10ASSESSMENT OF BRAIN SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Brain SOD was estimated spectrophotometerically (UV- 1800 ENG 240V; Shimadzu Coorporation, Japan) as described by Gupta and Sharma (2014a). This reaction is based on the reduction of NBT to water insoluble blue formazan.

2.11 ASSESSMENT OF BRAIN CATALASE ACTIVITY

The method of Luck (1971), which measures the breakdown of hydrogen peroxide, will be used to evaluate catalase activity. In a nutshell, 0.05 ml of the tissue homogenate supernatant and 3 ml of H2O2 phosphate buffer made up the assay combination. Using a spectrophotometer, the change in absorbance will be measured every 30 seconds for two minutes at 240 nm. The findings will be reported as micromoles of hydrogen peroxide broken down in milliseconds for each milligram of protein.

3.0 RESULTS

STATISTICAL ANALYSIS

Statistical analysis was done using Sigma Stat v3.5. All results were expressed as mean ± standard deviation. Data for the Morris water maze was statistically analyzed using three-way analysis of variance (ANOVA) followed by Bonferroni's post test. with both carotid ligation/without ligation X pharmacological treatments X days) and BCCAO were considered as factors. However, two-way ANOVA and Bonferroni's post test were used to statistically assess the data for all other parameters, taking into account factors such as drug treatment (BCCAO/without BCCAO X drug treatments).

3.1 ASSESSMENT OF LEARNING AND MEMORY USING MWM

3.1.1 EFFECT OF VARIOUS AGENT ON MEAN TIME SPENT IN THE QUADRANT (TSTQ) OF ANIMALS

A learning and memory impairment assessment was conducted using MWM, following the methods proposed by Sharma and Sharma (2013). Day 5 TSTQ on MWM was significantly lowered by BCCAO-induced CCH, indicating memory impairment or retrieval. Capsaicin (10 mg/kg to 30 mg/kg) treatment considerably reduced the learning and memory deficits brought on by BCCAO.

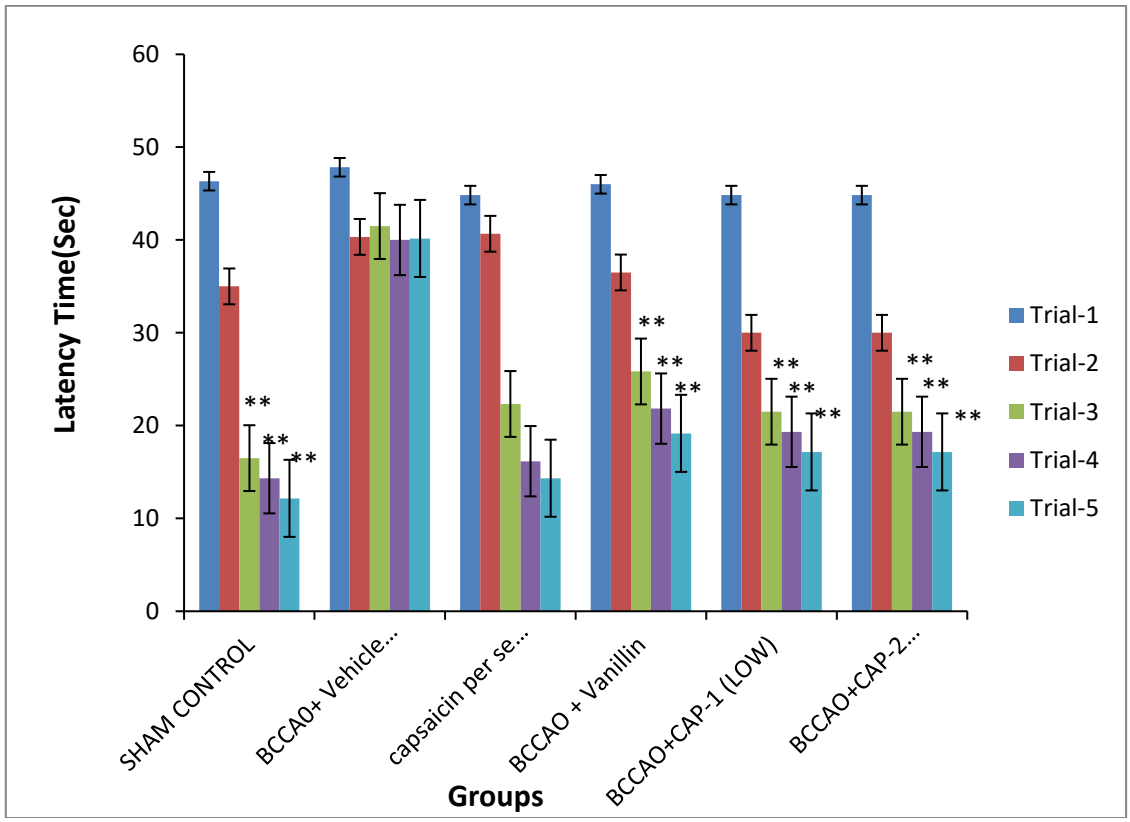


Fig. 1: Impact of different drugs on animals' mean time spent in the quadrant (TSTQ) using the Morris Water Maze

The three-way ANOVA results are presented as mean ± standard deviation, with Bonferroni's post test coming after. When compared Trial-1 with other trials within each group, F (2, 265) = 9026.800; p<0.001 versus TSTQ of the prior session; F (2, 265) = 1240.933; p <0.001 versus TSTQ of the corresponding day in the control group; F (12, 265) = 148.600; $ p <0.001 compared LT of the relevant day in the BCCAO group. CAP stands for capsaicin; BCCAO stands for bilateral common carotid artery occlusion; CAP-1 stands for low dosage and CAP-2 for high dose. SC stands for sham control.

3.1.2 IMPACT OF DIFFERENT AGENTS ON DAYS 1 AND 4 OF ANIMAL ESCAPE LATENCY TIME (ELT)

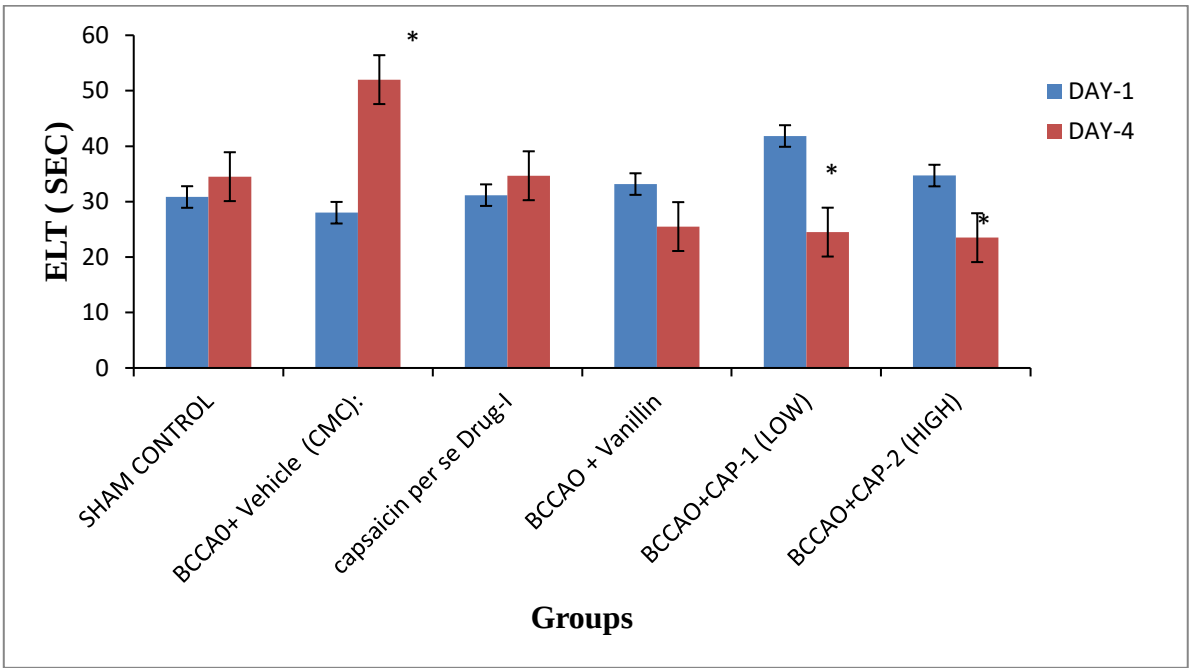


Fig. 2: Impact of different agents on the animals' day- 1 and day-4 escape latency times (ELT) utilizing the Morris water maze (MWM)

The three-way ANOVA results are presented as mean ± standard deviation, with Bonferroni's post test coming after. Within each group, F (3, 265) = 362.415; p <0.001 compared ELT of the corresponding day in the control group; F (12, 265) = 8.039; $ p <0.001 versus ELT of the respective day in the BCCAO group; and # p <0.001 versus ELT of the previous day within each group. CAP stands for capsaicin; BCCAO stands for bilateral common carotid artery occlusion; CAP-1 stands for low dosage and CAP-2 for high dose. SC stands for sham control.

3.1.3 USE OF ACTOPHOTOMETER TO DETERMINE THE EFFECT OF VARIOUS DRUGS ON LOCOMOTER ACTIVITY

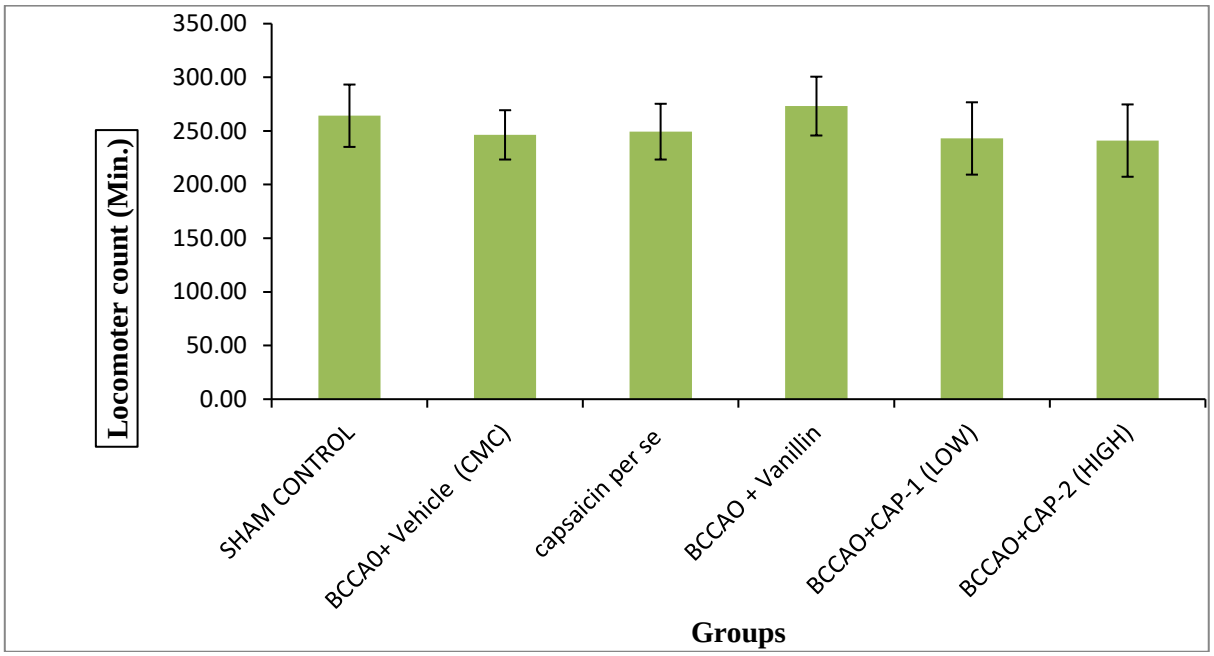


Fig. 3: Effect of various agents (TRPV-1 receptor) on locomoter activity by using actophotometer

5.1.4 DIFFERENT AGENTS' EFFECTS ON THE ACTIVITY OF ACETYLCHOLINESTERASE (ACHE) IN THE BRAIN

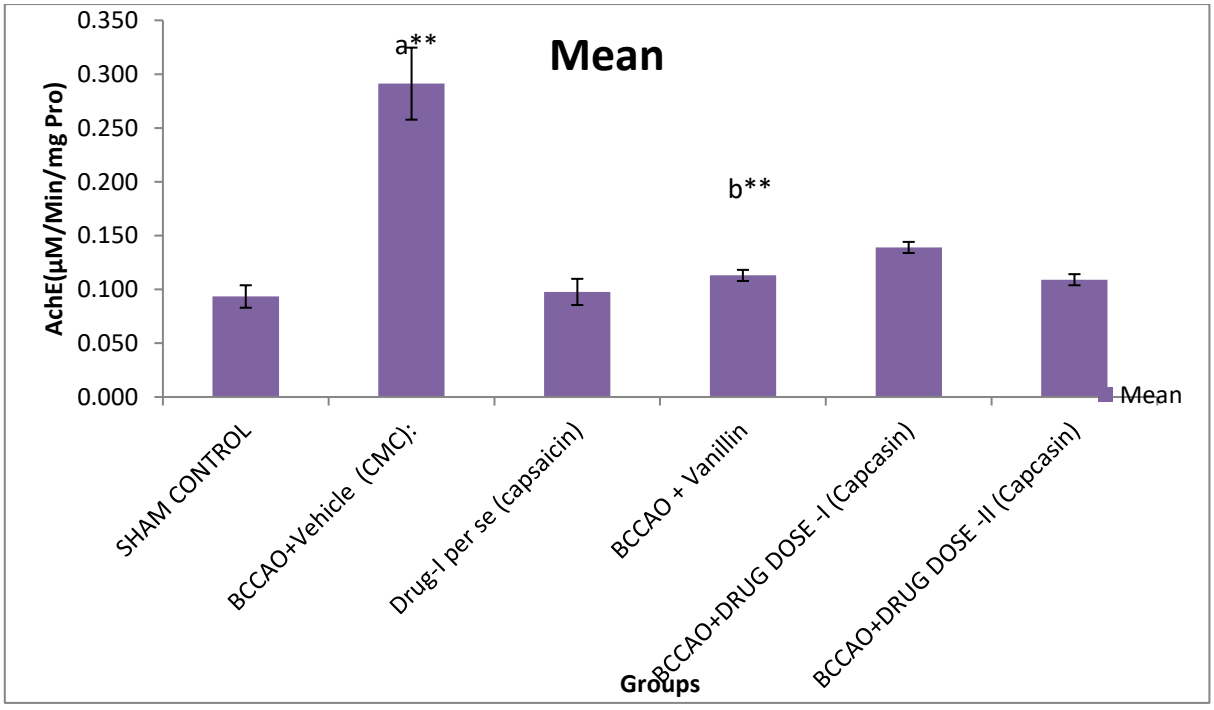


Fig. 4: Impact of Different Agents on (Ache) Acetylcholinesterases Activity in the Brain

Mean ± standard deviation is used to express the results of a two-way ANOVA, which is followed by a Bonferroni post test. F (1, 65) = 33.271; p<0.001 compared BCCAO group; F (2, 65) = 56817.800; *p<0. 001 versus sham control group (a). AChE stands for acetylcholinesterase; SC is for sham control; CMC for carboxymethylcellulose; CAP for capsaicin (10 mg/kg to 30 mg/kg); BCCAO for bilateral common carotid artery occlusion (b); CAP-1 for low dose and CAP-2 for high dose.

3.1.4 DIFFERENT THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) AND THEIR EFFECT ON THE MDA LEVEL IN THE BRAIN

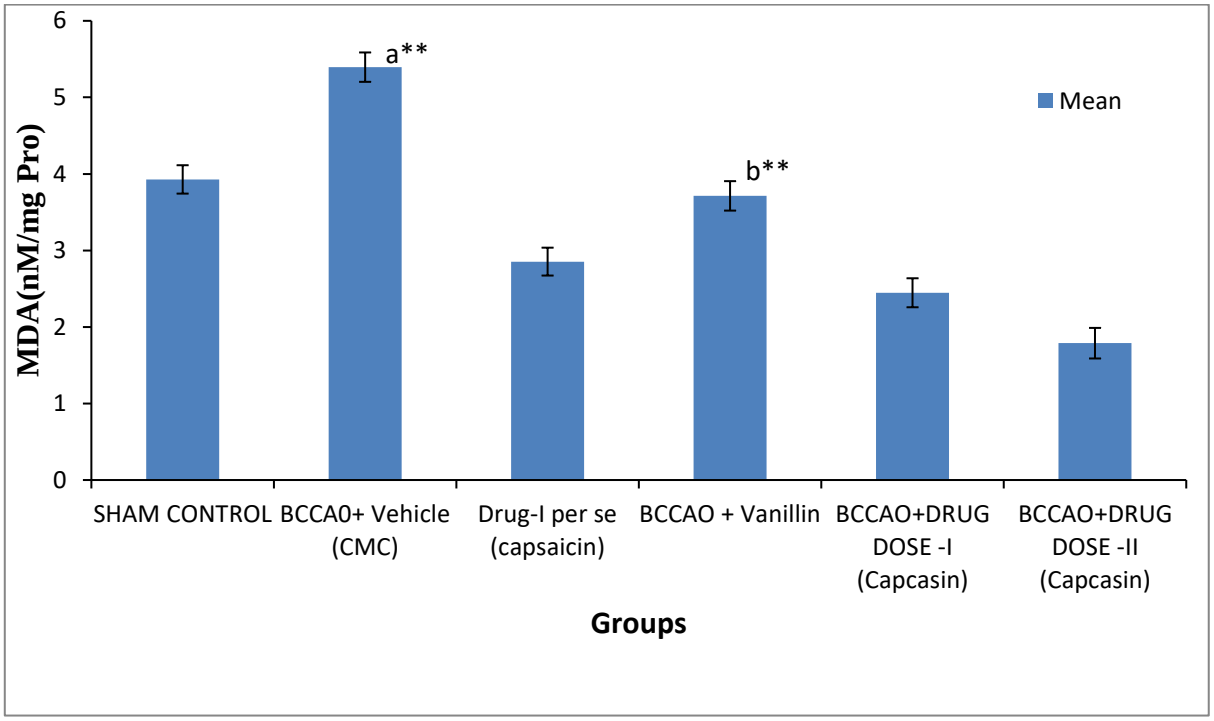


Fig. 5: Influence Of Different (Tbars) Thiobarbituric Acid Reactive Substance on the Level of (Mda) Malondialdehyde in the Brain

Mean ± standard deviation is used to express the results of a two-way ANOVA, which is followed by a Bonferroni post test. F (4, 65) = 43099.212; p<0.001 against the BCCAO group and F (2, 65) = 2022.652; p<0.001 against the sham control group. (a) The terms "sham control," "carboxymethylcellulose," "capcasin (10 mg/kg to 30 mg/kg), (b)"capsaicin," "BCCAO," "low dosage," "high dose," and "thiobarbituric acid reactive substances" (TBARS) are used.

3.1.5 IMPACT ON BRAIN SUPEROXIDE DISMUTASE AMOUNTS (SOD) ACTIVITY

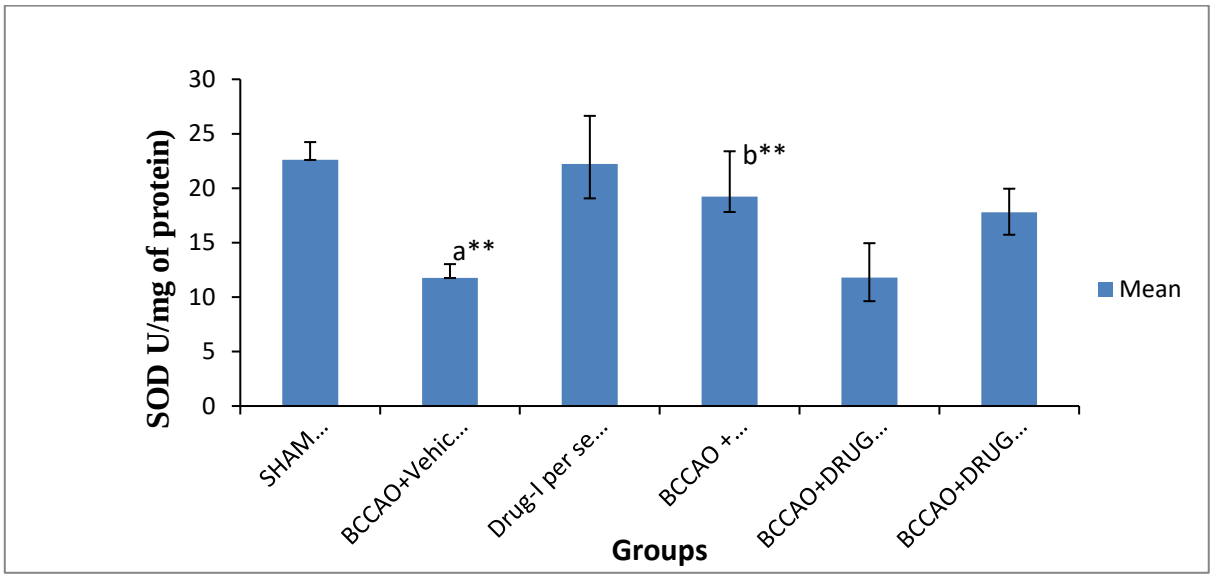


Fig. 6: Impact Of Different Substances On Brain Superoxide Dismutase (SOD) Inhibition

Mean ± standard deviation is used to express the results of a two-way ANOVA, which is followed by a Bonferroni post test. F (2, 65) = 225.477; p<0.001 compared BCCAO group; F (4, 65) = 8008.002; (a) * p<0. 001 versus sham control group. SOD is for superoxide dismutase; (b) BCCAO stands for bilateral common carotid artery occlusion; CAP stands for capsaicin (10 mg/kg to 30 mg/kg); and SC stands for sham control.

3.1.6 IMPACT ON GLUTATHIONE (GSH) IN THE BRAIN

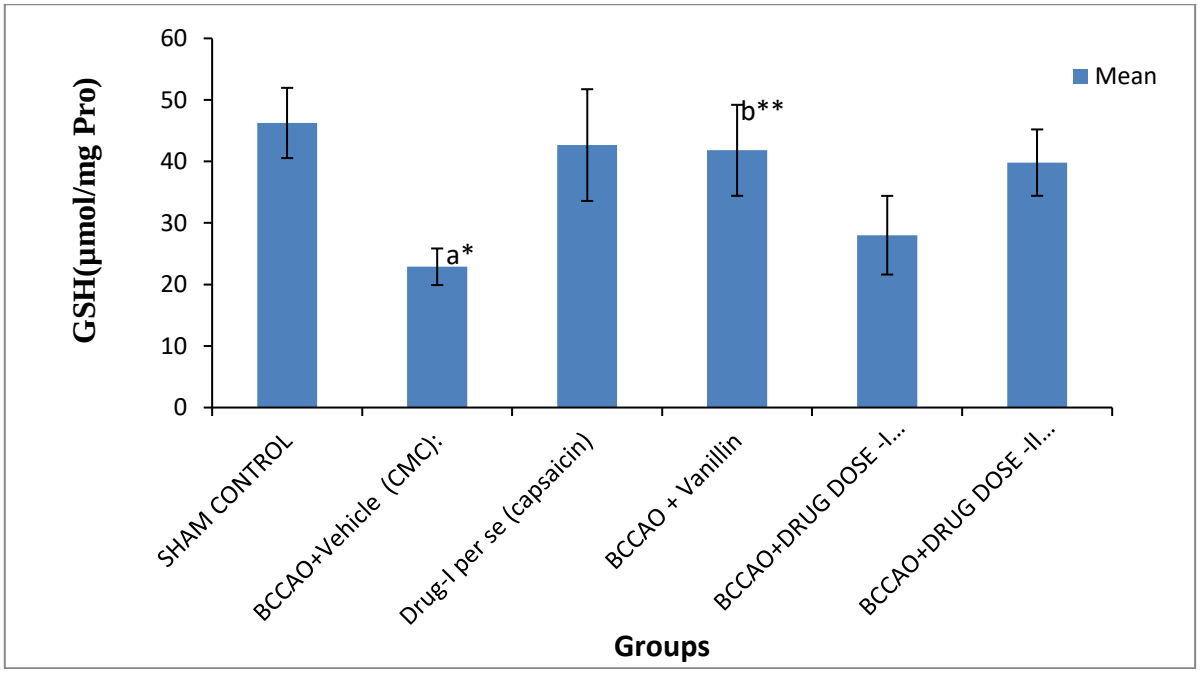


Fig. 7: Impact of Different Agents on Glutathione (GSH) In the Brain

Mean ± standard deviation is used to express the results of a two-way ANOVA, which is followed by a Bonferroni post test. F (2, 65) = 468.242; (a)* p<0.001 against the BCCAO group and F (1, 65) = 9.752 versus the sham control group. CAP stands for capsaicin (10 mg/kg to 30 mg/kg); (b) BCCAO stands for bilateral common carotid artery occlusion; CAP-1 stands for low dose; CAP-2 stands for high dose; and GSH stands for glutathione.

3.1.7 EFFECT OF BRAIN CATALASE ACTIVITY

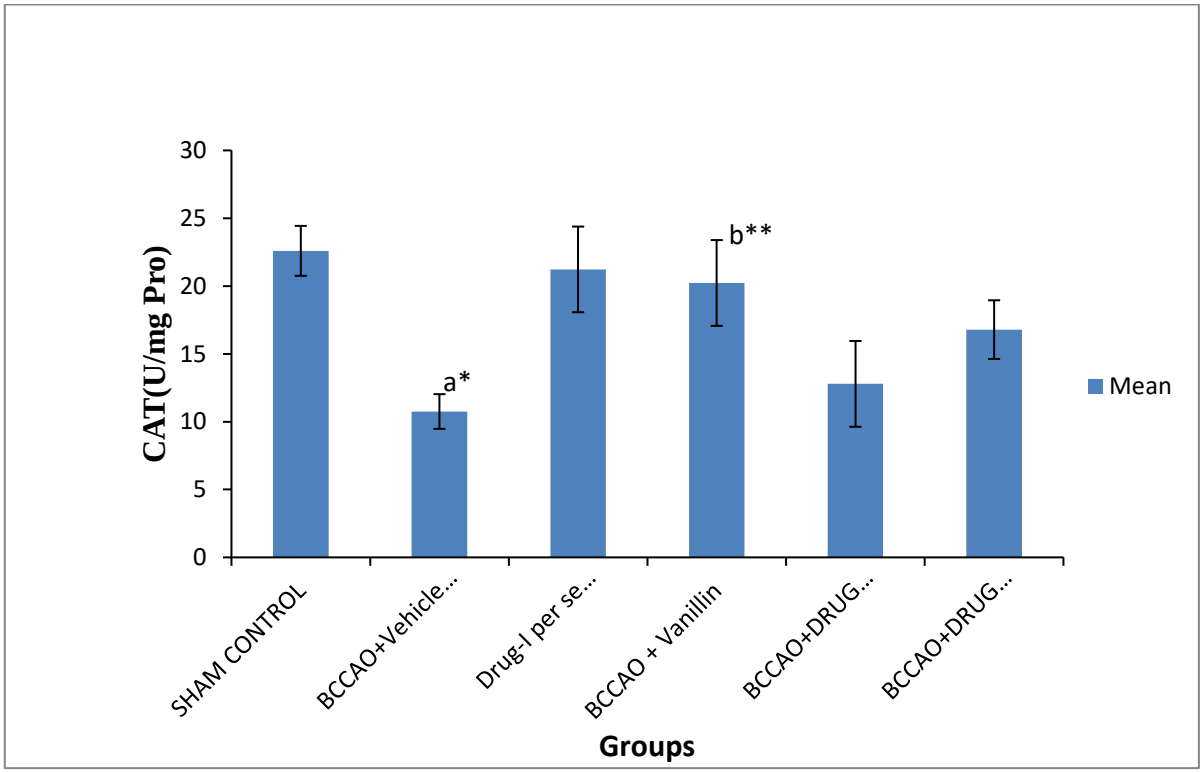


Fig. 8: Effect Of Brain catalase level

Mean ± standard deviation is used to express the results of a two-way ANOVA, which is followed by a Bonferroni post test. (a)In comparison to the sham control group, F (1, 65) = 68.24; * p<0.001; F (4, 65) = 7.552; p<0.001 BCCAO group. CAP stands for capsaicin; (b) BCCAO stands for bilateral common carotid artery occlusion; CAP-1 stands for low dosage; CAP-2 stands for high dose; catalase level; SC stands for sham control; TRPV-1 stands for vanilline; and CAP stands for capsaicin (10 mg/kg to 30 mg/kg).

3.1.8 INFARCT AREA %

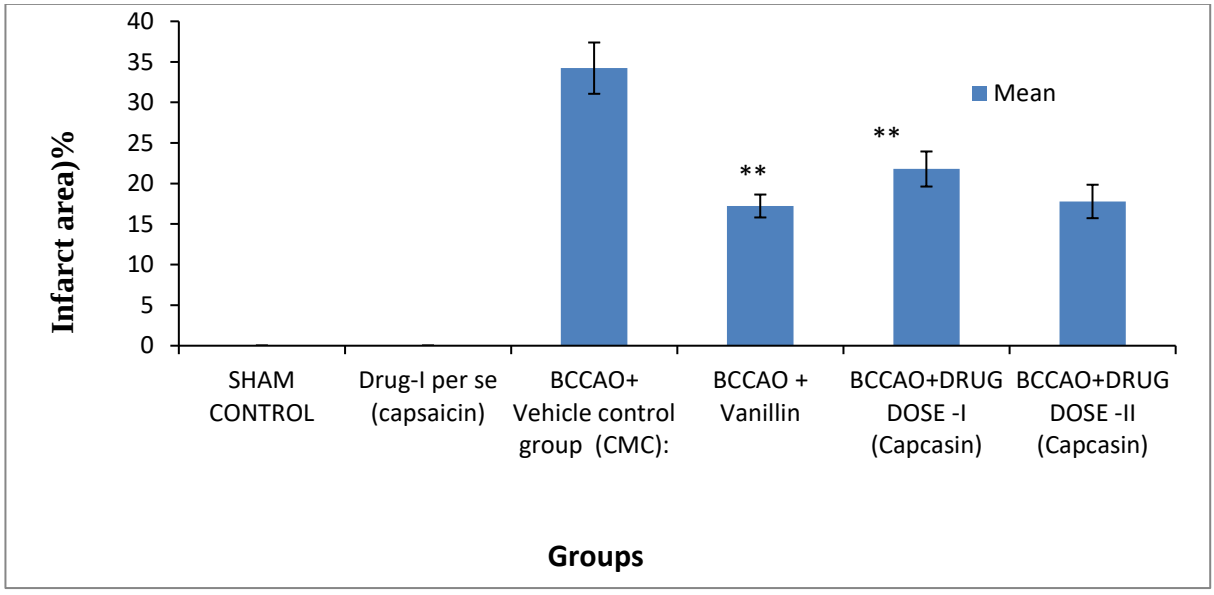


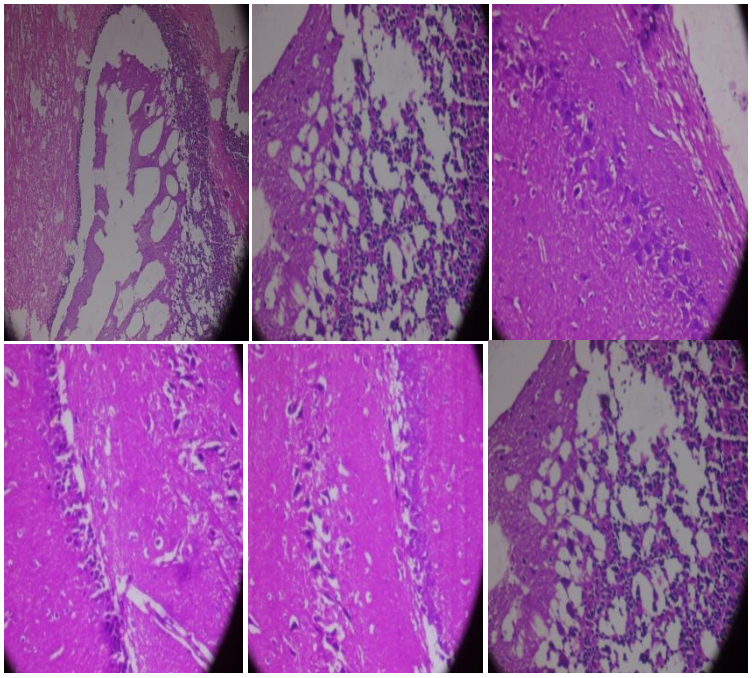
Fig 20(a): Effect on Infarct area%

Mean ± standard deviation is used to express the results of a two-way ANOVA, which is followed by a Bonferroni post test. (a) In comparison to the sham control group, F (1, 65) = 74.24; * p<0.001; F (4, 65) = 7.552; p<0.001 BCCAO

group. BCCAO: bilateral common carotid artery occlusion; SC: sham control group; CMC: carboxy-methylcellulose, treated groups shows statistically significant decrease in graph.

4.0EFFECTS OF VARIOUS DRUGS ON HISTOPATHOLOGICAL STUDIES

(a) control group, showing closely packed neuronal cells with vesicular nuclei; (b) vehicle control group group, showing slightly loosely packed neuronal cells (c) pyknotic nuclei chromatolytic cells group, showing increased number of normal neuronal cells with fewer pyknotic and chromatolytic cells;(d)Bilateral common carotid artery occlusion (e) Bccao+drug dose (low dose) group, showing greater number of normal closely packed neuronal cells along with very few number of pyknotic cells depicting beneficial effect of combination; (f) Bccao+drug dose (high dose) showing greater number of closely packed neuronal cells and very few chromatolytic cells.



Fig(8) Haematoxylin and Eosin (H&E) staining results obtained upon histological examination shown (H and E, 40xand 10x)

5.0 DISCUSSION AND CONCLUSION

In this study, permanent ligation of bilateral common carotid arteries has induced CCH, which causes learning and memory impairments as well as brain damage (high percent infarct) of animals. Furthermore, increased levels of brain MDA and AChE activity, with significantly reduced levels of brain GSH, SOD were observed in the brain of permanent ligation of animals. We examined the neuroprotective effects of capcasene on BCCAO-induced ischemia, which further leads to VaD.

Chlorogenic acid have attenuated ischemia induced learning and memory deficits, brain oxidative stress (GSH, SOD level), cholinergic dysfunction (AChE activity) and damage (infarct size). BCCAO is considered as a pathological cause in the induction of VaD, which is manifested by the cognitive decline and memory impairment (Galisova *et al*., 2014). In BCCAO we perform the permanent ligation of both common carotid arteries for studying the pathophysiology of VaD. While, performing the permanent ligation of bilateral common carotid arteries we found that the cerebral blood flow falls too sharply and significantly after the ligation of bilateral common carotid arteries, which leads to the generation of inflammatory elements (Sharma *et al.*, 2010), which have been reported to reduce the chances of survival up to a greater extent and thus higher mortality rate. By keeping the difference between the arterial occlusion, reduction in mortality rate has been achieved. Previous studies have suggested that BCCAO causes learning and memory impairment (Chen Y *et al*., 2006). Permanent ligation induced learning and memory deficits have been reported to be associated with decreased brain-derived neurotrophic factor (BDNF) expression and central level of acetylcholine (Sakar *et al*., 2014). Inhibition in the hippocampal region of the brain as well as oxidative stress (XiY *et al.*, 2014). Ligated animals have been reported to show increased brain AChE activity due to cholinergic dysfunction (Sakar *et al*., 2014). In the present study, BCCAO has induced hypoperfusion, which is further associated with learning and memory impairment. capsaicin participates in blockade of stress induced memory dysfunction and leads to activation of molecular mechanisms of memory retrieval after acquisition of learning (Yang *et al.*, 2006) have reported that activation of TRPV-1 receptor inhibits induction of LTP in hippocampus. (Chen Y *et al*., 2006).

In the present study, capsaicin improved hypoperfusion induced learning and memory deficits, which may be because of the above reasons. Free radical- induced neural damage has been implicated in cerebral hypoperfusion disorders (Galisova *et al*., 2014). It has been reported that BCCAO causes generation of reactive oxygen species (ROS), oxidative DNA damage as well as degeneration of hippocampal neurons. This oxidative injury to neurons impairs vascular function and neurovascular coupling, which results in a various cycle of further reduction of cerebral perfusion (Galisova *et al*., 2014). In our study, BCCAO induced hypoperfusion associated oxidative damage, which may be due to disturbance of the antioxidant enzyme status.

ACKNOWLEDGEMENTS

The author declare that there is no conflict of interest.

6.0 REFERENCES

1. Matthews FE, Arthur A, Barnes LE, Bond J, Jagger C, Robinson L, Brayne C. on behalf of the Medical Research Council Cognitive Function and Ageing Collaboration. A two decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of England: results of the Cognitive Function and Ageing Study I and II. Lancet. 2013 doi:10.1016/S01406736(13)615706. [PubMed] [Cross Ref]

2. World Health Organization. Dementia A Public Health Priority. World Health Organization; 2012.

3. Lezak,M.D.(1995). Neuropsychological Assessement (3ed.).New York: Oxford University Press.

4. American Psychiatric Association.(1994).Diagnostic and statistical manual of mental disorders (4 ed.).Washinton DC: American Psychiatric Association.

5. Zhao Y., Gu JH., Dai CL, et al. : Chronic cerebral hypoperfusion causes decrease of O GlcNAcylation, hyperphosphorylation of tau and behavioral deficits in mice. Front Aging Neurosci 2014;6:10.[PMC free article] [PubMed]

6. Bowler J. Modern concept of vascular cognitive impairment. Br Med Bull. 2007;83:291–305. [PubMed]

7. Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, Monsell SE, Kukull WA, Trojanowski JQ. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. Brain. 2013;136:2697–2706. [PMC free article] [PubMed]

8. Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S (2011) Vascular Contributions to Cognitive Impairment and Dementia. Stroke 42:2672-2713.

9. Farkas E, Luiten PGM, Bari F (2007) Permanent, bilateral common carotid artery occlusion in the rat: A model for chronic cerebral hypoperfusion-related neurodegenerative diseases. Brain Research Reviews 54:162-180.

10. Breitner JC. Dementia-epidemiological considerations, nomenclature, and a tacit consensus definition. J Geriatr Psychiatry Neurol. 2006;19(3):129–36 (PubMed)

11. American Psychiatric Association, editor. DSM-5. Diagnostic and statistical manual of mental disorders. 5th ed. Washington/DC: American Psychiatric Publishing; 2013.(PubMed)

12. World Health Organization (WHO). International statistical classification of diseases and related health problems, vol. 10th revision Geneva: WHO; 2011.

13. Sorbi S., Hort J., Erkinjuntti T., Fladby T., Gainotti G., Gurvit H., et al. (2012). EFNS –ENS Guidelines on the diagnosis and management of disorders associated with dementia. Eur. J.Neurol. 19, 1159–117910.1111/j.14681331.2012.03784.x [PubMed] [Cross Ref]

14. Roman, G. and Royall, D. (2004). A diagnostic dilemma: Is "Alzheimer's dementia" Alzheimer's disease, vascular dementia, or both *The Lancet Neurology, 3*.

15. Sadowski, M., Pankiewicz, J., Scholtzova, H., Yong-sheng, L., Quartermain, D., Duff, K., and Wisniewski, T. (2004). Links between the pathology of alzheimer's disease and vascular dementia. *Neurochemical Resaerch, 29*, 1257 – 1266.

16. Kalaria, R. (2002). Similarities between Alzheimer's disease and vascular dementia. *Journal of the Neurological Sciences, 203-204*, 29-34.

17. Watari, K. and Gatz, M. (2002). Dementia: A cross-cultural perspective on risk factors. *Generations. Diabetic Medicine, 18*, 355-359.

18. Mehta, N., and Khan, I. (2002). Cardiology's 10 greatest discoveries of the 20th century. *Texas Heart Institute Journal, 29*, 164-171.

19. Gorelick P. B., Scuteri A., Black S. E., Decarli C., Greenberg S. M., Iadecola C., et al. (2011). Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. Stroke 42, 2672–2713 10.1161/STR.0b013e3182299496 [PMC free article] [PubMed] [Cross Ref]

20. Hachinski V., Iadecola C., Petersen R. C., Breteler M. M., Nyenhuis D. L., Black S. E., et al. (2006).National Institute of Neurological Disorders and StrokeCanadian Stroke Network vascular cognitive impairment harmonization standards. Stroke 37, 2220–224110.1161/01.STR.0000237236.88823.47[PubMed] [Cross Ref]

21. Jellinger K. A. (2013). Challenges in the neuropathological diagnosis of dementias. Int. J.Neuropathol. 1, 8–52.

22. Jellinger K. A. (2007b). The enigma of mixed dementia. Alzheimers Dement. 3, 40–53 10.1016/j.jalz.2006.09.002 [PubMed] [Cross Ref]

23. Emilien, G rard, et al. Alzheimer Disease: Neuropsychology and Pharmacology. Basel: Birkhauser, 2004.

24. Xu Y, Zhang JJ, Xiong L, Zhang L, Sun D, Liu H. Green tea polyphenols inhibit cognitive impairment induced by chronic cerebral hypoperfusion via modulating oxidative stress. JNutr Biochem. 2010.

25. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med.2004.

26. Kitamura A, Fujita Y, Oishi N, Kalaria RN, Washida K, Maki T, Okamoto Y, Hase Y,Yamada M, Takahashi J, Ito H, Tomimoto H, Fukuyama H, Takahashi R, Ihara M. Selective white matter abnormalities in a novel rat model of vascular dementia. Neurobiol Aging. 2012.

27. Choi JY, Morris JC, Hsu CY. (1998) Aging and cerebrovascular disease. Neurol Clin 16:687-711

28. Levy BI. (2001) Artery changes with aging: degeneration or adaptation? Dialogues in Cardiovascular Medicine 6:104 – 111

29. Pantoni L. (2002) Pathophysiology of age-related cerebral white matter changes. Cerebrovasc Dis 13:7-10

30. Baltan S, Besancon EF, Mbow B, Ye Z, Hamner MA, Ransom BR. (2008) White Matter Vulnerability to Ischemic Injury Increases with Age Because of Enhanced Excitotoxicity. J Neurosci 28:1479-1489

31. Gupta S, Sharma B. Pharmacological modulation of I(1)- imidazoline and 2 adrenoceptors in sub acute brain ischemia induced vascular dementia. Eur J Pharmacol 2014a; 723:80-90.

32. Medina AE. Therapeutic utility of phosphodiesterase type I inhibitors in neurological conditions. Front Neurosci 2011; 5: 21.

33. Portelius E, Zetterberg H, Skillbäck T, Törnqvist U, Andreasson U, Trojanowski JQ, Weiner MW, Shaw LM, Mattsson N, Blennow K *et al* (2020) Cerebrospinal fluid neurogranin:

relation to cognition and neurodegeneration in Alzheimer's disease. Brain J Neurol 138:3373–3385 Abstract/FREE Full Text Google Scholar

34. Galisova A, Baciak L, Jozefovicova M, *et al*. Pathophysiological rat model of vascular dementia: magnetic resonance spectroscopy, microimaging and behavioral study. Brain Res 2014; 1568: 10-20.

35. Du J, Ma M, Zhao Q, *et al*. Mitochondrial bioenergetic deficits in the hippocampi of rats with chronic ischemia-induced vascular dementia. Neurosci 2013; 231: 345-52.

36. Sharma B, Singh N. Pitavastatin and 4'-hydroxy-3'-methoxyacetophenone (HMAP) reduce cognitive dysfunction invascular dementia during experimental diabetes. Curr Neurovasc Res 2010; 7(3): 180-91.

37. Gupta S, Sharma B. Pharmacological modulation of I(1)-imidazoline and alpha2-adrenoceptors in sub acute brain ischemia induced vascular dementia. Eur J Pharmacol 2014a; 723:80-90.

38. Sakr HF, Khalil KI, Hussein AM, Zaki MS, Eid RA, Alkhateeb M.Effect of dehydroepiandrosterone (DHEA) on memory and brain derived neurotrophic factor (BDNF) in a rat model of vascular dementia. J Physiol Pharmacol 2014; 65(1): 41-53.

39. Xi Y, Wang M, Zhang W, *et al*. Neuronal damage, central cholinergic dysfunction and oxidative damage correlate with cognitive deficits in rats with chronic cerebral hypoperfusion. Neurobiol Learn Mem 2014; 109: 7-19.

40. Chen Y, Mancuso J, Zhao Z, *et al*. Vasodilation by in vivo activation of astrocyte endfeet via two-photon calcium uncaging as a strategy to prevent brain ischemia. J Biomed Opt 2013; 18(12):126012.

41. Fotuhi M, Hachinski V, Whitehouse PJ. Changing perspectives regarding late-life dementia. Nat Rev Neurol. 2009;5:649–58.

42. de La Torre JC. Is Alzheimer’s disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neurol. 2004;3:184–90.

43. Chui H. Vascular dementia, a new beginning: shifting focus from clinical phenotype to ischemic brain injury. Neurol Clin. 2000;18:951-978.

44. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA (2011). Microinfarct pathology, dementia, and cognitive systems. Stroke doi:10.1161/STROKEAHA.110.595082 PubMedPubMedCentral

45. Bink DI, Ritz K, Aronica E, van deer Weerd L, Daemen MJ (2013) Mouse models to study the effect of cardiovascular risk factors on brain structure and cognition. J Cereb Blood Flow Metab. doi:10.1038/jcbfm.2013.140PubMedPubMedCentral.

46. Isingrini, E., Camus, V., le Guisquet, A. M., Pingaud, M., Devers, S., & Belzung, C. (2010). Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: A model of fluoxetine resistance in mice. PLoS ONE, 5(4). https://doi.org/10.1371/journal.pone.0010404

47. 16. Jollow D.J., G. J. R. (1974). Bromobenzene-induced Liver Necrosis. Protective role of Glutathione and Evidence for 3,4-Bromobenzene oxide as the hepatotoxic metabolite. Pharmacology.

48. 17. Köhler, O., E. Benros, M., Nordentoft, M., Farkouh, M. E., Iyengar, R. L., Mors, O., & Krogh, J. (2014). Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects a systematic review and meta-analysis of randomized clinical trials. In JAMA Psychiatry (Vol. 71, Issue 12, pp. 1381–1391). American Medical Association. https://doi.org/10.1001/jamapsychiatry.2014.1611

49. 18. Kolla, N., Wei, Z., Richardson, J. S., & Li, X. M. (2005). Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide. Journal of Psychiatry and Neuroscience, 30(3), 196–201.

50. 19. Mandal S, Jaiswal V, Sagar MK, Kumar S. Formulation and evaluation of carica papaya nanoemulsion for treatment of dengue and thrombocytopenia. Plant Arch. 2021;21:1345-54.