

RESEARCH ARTICLE

Neuroprotective Potential of Indian Propolis and in Combination with Curcumin against Bilateral Common Carotid Artery Occlusion Induced Vascular Dementia in Rats

Meenu^{1*}, Tarun Virmani¹, Lubhan Singh²

¹School of Pharmaceutical Sciences, Modern Vidya Niketan University, Palwal, Haryana, India

²Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

Received: 23rd July, 2022; Revised: 24th August, 2022; Accepted: 08th September, 2022; Available Online: 25th September, 2022

ABSTRACT

Vascular dementia (VaD) is associated with chronic brain ischemia and gradual memory loss which is increasing gradually but limited treatments are available as therapeutic moieties to combat the VaD. Hence in this study Indian propolis alone or in combination with curcumin was explored as a treatment modality to alleviate the VaD. VaD model was induced by bilateral common carotid artery occlusion (BCCAO). Indian propolis (200 mg/kg), curcumin (200 mg/kg) individually, and the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) were administered in BCCAO model followed by an estimation of behavioral (neurological score, hanging and open field test) and biochemical parameters (reduced glutathione, catalase, malondialdehyde, and acetylcholine esterase). In this study it was found that there were no changes in the locomotor activity while the neurological score was reduced and the hanging time was increased in the drug treated group which clearly depicts the neuroprotective potential of curcumin, Indian propolis individually and the combination of Indian propolis with curcumin. The combination of Indian propolis with curcumin was much better in terms of neuroprotective activity than Indian propolis and curcumin treated group as depicted by the results of behavioural and biochemical parameters. Moreover, haematoxylin and eosin (H and E) staining was performed, and was found that a combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) attenuated better histological alterations of the hippocampus in BCCAO model group than in other treated groups. Hence these findings provided that Indian propolis in combination with curcumin has great potential as a neuroprotective agent on the cognitive deficits of VaD rats.

Keywords: Curcumin, Hematoxylin and eosin (H and E) staining, Indian propolis, Vascular dementia.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.3.64

How to cite this article: Meenu, Virmani, T, Singh, L. Neuroprotective Potential of Indian Propolis and in Combination with Curcumin against Bilateral Common Carotid Artery Occlusion Induced Vascular Dementia in Rats. International Journal of Drug Delivery Technology. 2022;12(3):1319-1326.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Vascular dementia (VaD) is one of the second major causes of dementia after Alzheimer's disease, accounting for about 15% of cases.¹ The treatment available for most types of dementia provides only symptomatic relief² but no curative treatment is available.³ Various therapeutic moieties for vascular dementia are under clinical trial and no FDA-approved drug moiety is available to treat VaD till now.⁴ Some herbal and synthetic drugs namely α -mangostin,⁵ diosgenin derivative,⁴ *Ginkgo biloba*, sailuotong,⁶ memantadine,⁷ donepezil,⁸ huperzine A,⁹ curcumin,¹⁰ *Panax ginseng*, *Crocus sativus*,¹¹ *Bacopa monnieri*¹² have been identified by various researchers to alleviate the symptoms of VaD but still, it needs more exploration hence it was selected for the present research.

Due to the inherent safety issues, medications of natural origin are superior to those of synthetic or semi-natural origin.¹³ Hence Indian propolis, resin like derivative was used to provide neuroprotective effect in VaD. Propolis is an intricate natural resin mix of honeybees derived from substances extracted from fruit, bud and exudate compounds.¹⁴ Various activities have been reported related to Indian propolis as anticancer,¹⁵ antimicrobial,¹⁶ anti-inflammatory,¹⁷ antioxidant agent,¹⁸ and improvement of oral health.¹⁹ But it has not been identified as a treatment option for VaD hence in the present study; it was explored as a neuroprotective agent in VaD. It was used individually as well as in combination with curcumin to provide a synergistic effect in VaD. Curcumin, a polyphenol that is abundantly present in the rhizome of the turmeric plant

*Author for Correspondence: meenu.bhati@mvn.edu.in

(*Curcuma longa*), has the potential to cure major portions of the diseases like neurodegenerative disorder,¹⁰ cancer,²⁰ diabetes,²¹ bacterial infection,²² gouty arthritis²³ and has been used in combination with various synthetic as well as herbal drug moieties to provide a synergistic effect in the disease amongst some combination have been commercialized. To identify the potential of Indian propolis as a neuroprotective agent disease model has to be induced. For the induction of VaD various models, vessel occlusion model (2-vessel occlusion/Bilateral common carotid artery occlusion model, 4-vessel occlusion, and unilateral common carotid artery occlusion), multiple infarcts and thromboembolism model, High fat diet model, Models using risk factors to induce VaD via diabetes and hypertension have been used but in the present study bilateral common carotid artery occlusion (BCCAO) was used for the induction of VaD because it reduces about 30–45% of blood in the cortex and 20% in the hippocampus cerebral of rat's brain.²⁴ BCCAO results the increase in oxidative stress, inflammation, glial activation, striatal infarction, hippocampal (CA1) and cortical neuronal damage leading to the cognitive impairment.²⁵

MATERIALS AND METHODS

Plant and Material

Dried whole extract of the substance Indian propolis and curcumin (Chemical constituent of *C. longa*) were purchased from Vital Herbs, Z-26/27 Commercial Enclave, Mohan Garden Uttam Nagar, Delhi-110059. The batch no. for Indian propolis and curcumin were VH/PE/VHP012, VH/CE/VHC213 respectively. The bulk density for Indian propolis and curcumin was same *i.e.*, 49.2 g/100 mL.

Drug and Chemicals

1-Chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), 5, 5- dithiobis-2-nitrobenzoic acid (DTNB), 2, 3, 5-triphenyltetrazolium chloride (TTC), thiobarbituric acid (TBA), nitroblue tetrazolium (NBT), paraformaldehyde, acetylthiocholine iodide (AcSCh), epinephrine and trichloroacetic acid (TCA) were used. All the other chemicals used were of analytical grade.

Ethical Approval

The experiments were performed as per protocols set by CPCSEA, New Delhi, India which was duly approved by Institutional Animal Ethical Committee (Approval No. 1352/PO/Re/S/10/CPCSEA).

Experimental Animals

Adult male wistar rats weighed between 220–270 g were used. Rats were kept in conventional lab conditions, maintained at 25 ± 3°C temperature and relative humidity of 45 to 55% with 12 hours light: 12 hours dark cycle. Before the surgery, food was withheld for 12 hours. All procedures were carried out in line with the Ministry of animal welfare division, Government and control and supervision of experiments on animals (CPCSEA) guidelines Government of India, New Delhi.

Surgical Procedure for BCCAO

The animals were anesthetized with ketamine (80 mg/kg, i.p.) followed by xylazine (5 mg/kg i.p.). Atropine sulphate (0.1 mg/kg, i.p.) was given to prevent respiratory distress. After that, the bilateral carotid arteries were obstructed by ligation for 10 minutes, and then unrestricted for 10 minutes, and this cycle was repeated three times. The threading was removed and the incision closed. After induction of 21 days, the rats were killed by decapitation, and their brains were collected and processed.²⁶

Post-operative Care

The animals were kept in a maintained temperature at 25 ± 3°C in individual cages after the surgery and recovery of anesthesia until they gained full consciousness, and then they were kept together in a group of 3 animals per cage. Food and lithium water was kept inside the cage for 24 hours so that the animal could easily access it.

Experimental Groups and Treatment Schedule

Animals were divided into six groups. The first group, control group (n=8) (animals were put in control without any surgical procedure), and the second group was sham-operated (n=8) (animals were subjected to surgical procedure, but did not occlude BCCAO) received vehicle (0.1% tween 80) third was BCCAO group only (n=6); occluded the bilateral arteries, fourth was BCCAO group treated with curcumin (n=6) received curcumin (200 mg/kg in 0.1% tween 80, orally); fifth was BCCAO group treated with Indian propolis (200 mg/kg in 0.1% tween 80, orally) (n=6); and sixth was BCCAO group treated with the combination of Indian propolis (100 mg/kg 0.1% tween 80) with curcumin (100 mg/kg in 0.1% tween 80, orally) (n=06). Vehicle or drugs were fed once daily for 21 days after the experimental procedure. After the completion of 21 days, animals were assessed for neuro-behavioural assessment and then sacrificed for biochemical analysis and H and E staining.

Neuro-behavioural Assessment

The assessment of neuro-behavioural performance was done with the help of parameters, a neurological scale, a hanging test, and an open field test. These parameters generally check the extent of impaired consciousness, muscle strength, and gross locomotor activity among all model and treated groups.

Neurological Function

A five-point neurological scale was used to assess neuro-behavioural functioning across all experimental groups after the 21 days course of treatment: Rats with no other neurological deficits showed score=0, rats extended both forelimbs toward the floor when gently suspended 1m above the floor and with no other signs of neurological deficit; (a) rats consistently flexed the forelimb contralateral to BCCAO; (b) rats circled toward the contralateral side when the tail was pulled; (c) rats spontaneously circled toward the contralateral side when allowed to move freely; and (d) no spontaneous movement with an apparent depressed level of consciousness.²⁷

Hangin Test

The assessment of muscle strength of all experimental model groups was done using hanging test. At the beginning of process, rats were placed standing on a wire mesh platform. The platform was inverted and positioned 750 mm above a landing box filled with wood shavings to protect the rat from any injuries from the fall. The experimenter briefly moved the platform to get the rat to hold onto the bars. The length of time that the rat was able to hang from the inverted platform was recorded. If the duration of the 90 seconds was reached, then the trial was ended and a time was recorded, the average time across the trials at each time point used for evaluation.²⁸

Open Field Test (OFT)

OFT is frequently used to check the locomotor activity, exploratory activity, and anxiety-like response in experimental animals. After the ischemic insult, the animal was placed at one of the corners in the open arena of a 40 × 40 cm box for 5 minutes and the duration was measured.²⁹

Biochemical Analysis

All animals received ethyl ether anesthesia and phosphate buffer saline perfusion on day 22. Animals were sacrificed by decapitation, and the brains were then extracted after blood was removed from brain tissue using perfusion. Brain tissue was rinsed with ice-cold isotonic saline and the hippocampus was separated and weighed. The rat hippocampus tissues were then homogenized in a volume 10 times the tissue's weight with ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 minutes (4°C), and a part of the supernatant was used for biochemical analysis.³⁰

Estimation of Total Protein in the Brain

The total protein content in tissue homogenates was measured following the method of Lowry et al. using bovine serum albumin as a standard.³¹

Measurement of MDA in Brain Homogenate

Thiobarbituric acid reactive substance (TBARS) concentration was calculated as a measure of lipid peroxidation. Tris-HCl buffer (pH 7.4) and 0.5 mL of the brain were mixed and the mixture was 2 hours at 37°C. Then, 1-mL of 10% ice-cold trichloroacetic acid (TCA) was added to the above solution. Furthermore, 1-mL of 0.67% thiobarbituric acid (TBA) solution was mixed with 1-mL of supernatant, after centrifugation at 2000 g for 20 minutes. The test tubes were placed in boiling water for 10 minutes allowed to cool, and 1-mL double distilled water (DDW) was added. The malondialdehyde (MDA) was analyzed by reaction with thiobarbituric acid at 532 nm.³²

Measurement of Reduced Glutathione (GSH) in Brain Homogenate

Ellman's (1959) method was used for the estimation of GSH. For the separation of protein, brain homogenate was mixed with 10% TCA and centrifuged. 2 mL of phosphate buffer (pH 8.4), 0.5 mL of DTNB, and 0.4 mL double distilled water were added to 0.01 mL of the supernatant. The mixture was vortexed and the absorbance was measured at 412 nm.³³

Measurement of Catalase (CAT) in Brain Homogenate

Catalase activity was assessed by incubating the enzyme sample in a 1.0 mL substrate (65 mmol/mL hydrogen peroxide in 60 mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37°C for three minutes. The reaction was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide was taken at 374 nm against the blank.³⁴

Acetylcholinesterase (AChE) Activity

In the hippocampus and cortex, acetylcholinesterase (AChE) activity was estimated and presented as $\mu\text{mol}/\text{min}/\text{g}$ of brain.³⁵ The reaction mixture (2 mL final volume) contained 100 mM K⁺-phosphate buffer, pH 7.5, and 1-mM 5,5-dithiobisnitrobenzoic acid (DTNB). The procedure relies on the synthesis of the 5, 5-dithio-bis-acid-nitrobenzoic yellow anion, which is detected by absorbance at 412 nm after a 2-minutes incubation at 25°C. Pre-incubation of an enzyme (40–50 μg of protein) for 2 minutes was done. By adding the 0.8 mM acetylthiocholine iodide (AcSCh), the reaction was initiated. All samples were run in duplicate or triplicated.³⁶

Histopathology

The brain tissues of rats were removed after the sacrifice and the incubation in 4% paraformaldehyde was done 24 hours at 4°C. The entire hippocampus was among the brain samples extracted from beneath the optic chiasm to embed in paraffin. For histological investigation, embedded brain tissue sections were continuously cut into 4–5 μm sections as directed. The histomorphology of neurons was examined using light microscopy, and hippocampal CA1 and CA3 subfields were chosen and counted at a 400X magnification to look for morphological changes.³⁷

Statistical Analysis

The values were expressed as mean \pm S.E.M. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test with $p \leq 0.05$ were considered as significant.

RESULT

Effect of Neurological Function on Experimental Groups

The neurological score was used to check the neurological function of experimental models. The control and sham-operated rats, showed no neurological deficit having a score=0, while in BCCAO group caused a marked change in the behavior of the animals and exhibited a severe neurological deficit, the score was (4.16 \pm 0.30) and showed circling towards the contralateral side and had reduced mobility when compared to the control and sham-operated animals. The animals in the curcumin (200 mg/kg), Indian propolis (200 mg/kg) and Indian propolis with curcumin (100 mg/kg) treated group followed to BCCAO improved the neurological deficit; score: 2.66 \pm 0.21, 2 \pm 0.25 and 0.66 \pm 0.33, respectively. The rats in the Indian propolis with curcumin (100 mg/kg) treated group followed to BCCAO showed significant ($p < 0.001$) improvement in

Table 1: Effect of Indian propolis (200 mg/kg), Curcumin (200 mg/kg), and combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) on neurological score, hanging performance, open field test of rats subjective to BCCAO.

Neurobehavioral parameter	Control	Sham	BCCAO	BCCAO+Curcumin (200 mg/kg)	BCCAO+Indian propolis (200 mg/kg)	BCCAO+Indian propolis (100 mg/kg)+Curcumin (100 mg/kg)
Neurological score	0	0	4.16 ± 0.30a***b***	2.66 ± 0.21a***b***c***	2 ± 0.25a***b***c***	0.66 ± 0.33c***d***e**
Hanging test (fall-off-time) sec.	52.83 ± 4.60	32.16 ± 4.86a*	10.83 ± 2.73a***b*	30.13 ± 3.33a***	31.66 ± 5.16a*c*	33.83 ± 3.80a*c**
Open field test (locomotor counts)	48.3 ± 4.8	46.8.33 ± 6.6	47.2 ± 7.5	54.0 ± 3.1	55.0 ± 4.6	52.5 ± 5.9

Table 2: Effect of Indian propolis (200 mg/kg), Curcumin (200 mg/kg), and combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) on MDA, GSH, CAT and AChE of rats subjective to BCCAO.

Biochemical parameter	Control	Sham	BCCAO	BCCAO+ Curcumin (200 mg/kg)	BCCAO+ Indian propolis (200 mg/kg)	BCCAO+ Indian propolis (100mg/kg)+ Curcumin (100 mg/kg)
MDA (nM/mg Protein)	0.64 ± 0.28	0.663 ± 0.191	6.335 ± 0.595a***b***	2.66 ± 0.257a***b***c***	1.41 ± 0.37 c***	0.98 ± 0.246c**d*
GSH (µM/mg Protein)	46.99 ± 4.34	45.40.33 ± 2.24	11.71 ± 1.75b***	34.33 ± 4.11c*	39.26 ± 6.50c**	44.51 ± 6.39c***
Catalase (U/mg Protein)	28.27 ± 2.17	26.18 ± 3.01	10.34 ± 1.26a***b***	16.73 ± 1.13a***	21.35 ± 2.30c*	25.40 ± 2.63c**
AChE (µM/Min/mg Protein)	0.060 ± 0.015	0.069 ± 0.007	0.224 ± 0.039a***b***	0.128 ± 0.023c**	0.095 ± 0.17c***	0.080 ± 0.013c***

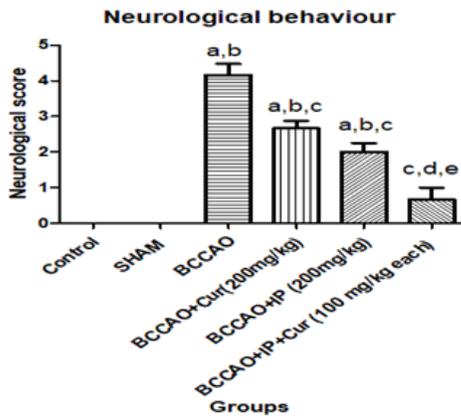


Figure 1: Values are expressed as mean ± SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison test. Pa < 0.05 compared to Control, pb < 0.05 compared to sham, pc < 0.05 compared to BCCAO, pd < 0.05 compared to BCCAO+CUR, pe < 0.05 compared to BCCAO+IP, respectively.

the behavior when compared to the BCCAO rats as shown in Figure 1 (Table 1).

Effect of Hanging Performances on Experimental Groups

Indian propolis (200 mg/kg) and the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) treated group showed highly significant wire gripping strength and latency time (23.33 ± 3.04 , $p < 0.05$), (33.83 ± 3.80 , $p < 0.05$), respectively as compared to BCCAO model group (10.83 ± 2.73 , $p < 0.001$). BCCAO treated rats showed significant ($p < 0.001$)

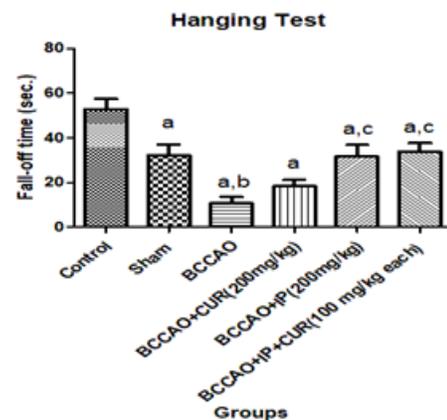


Figure 2: Values are expressed as mean ± SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison tests. Pa < 0.05 compared to Control, pb < 0.05 compared to sham, pc < 0.05 compared to BCCAO, respectively.

reduction in latency time and gripping strength as compared with control (52.83 ± 4.60) and Sham treated group (32.16 ± 4.86) (Figure 2 and Table 1).

Effect of Open Field Test on Experimental Groups

All groups did not affect the motor performances in the open field test, as the rats of the BCCAO induced groups, Indian propolis (200 mg/kg), curcumin (200 mg/kg), combination of Indian propolis (100 mg/kg) with curcumin group (100 mg/kg) travelled almost similar distance, showed normal mobility (47.2 ± 7.5), (55.0 ± 4.6), (54.0 ± 3.1), and (52.5 ± 5.9)

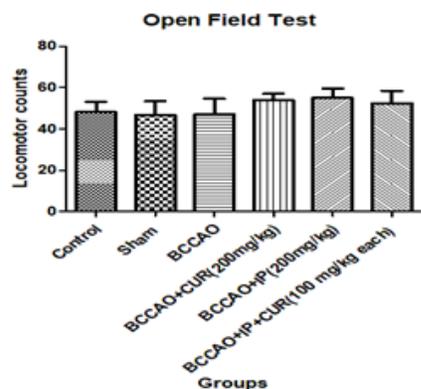


Figure 3: Values are expressed as mean ± SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are not statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison tests.

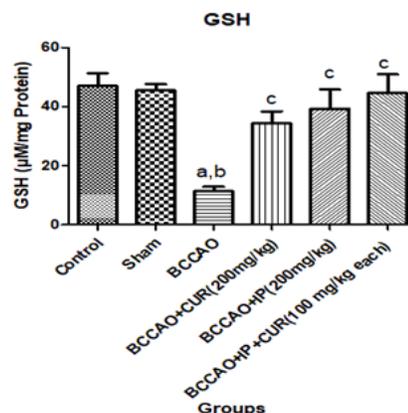


Figure 5: Values are expressed as mean ± SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison tests. Pa < 0.05 compared to Control, pb < 0.05 compared to sham, pc < 0.05 compared to BCCAO, respectively.

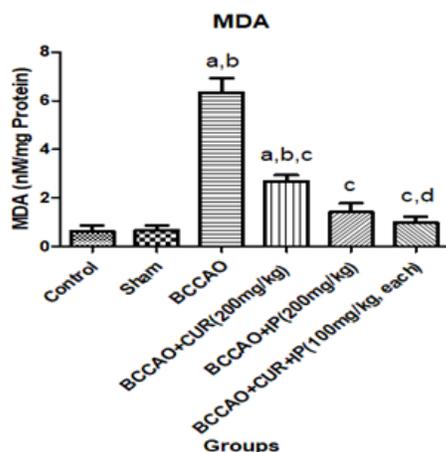


Figure 4: Values are expressed as mean ± SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison test. Pa < 0.05 compared to Control, pb < 0.05 compared to sham, pc < 0.05 compared to BCCAO, pd < 0.05 compared to BCCAO+CUR, respectively.

in comparison to control groups (48.3 ± 4.8) and sham, respectively. There are no significant differences among the all groups depicted in Figure 3 and Table 1.

Effect of Experimental Groups on Tissue Malondialdehyde (MDA)

On the basis of statistical analysis it was observed that the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) treated group showed statistical highly significant decrease in the malondialdehyde (MDA) (0.98 ± 0.24 , $p < 0.001$) as compared to BCCAO model (6.33 ± 0.595 , $p < 0.01$). BCCAO treated rats showed highly significant increase in the MDA (6.33 ± 0.595 , $p < 0.001$) as compared with control (0.64 ± 0.28 , $p < 0.001$) and sham treated group (0.663 ± 0.191 , $p < 0.001$) as shown in Figure 4 and Table 2.

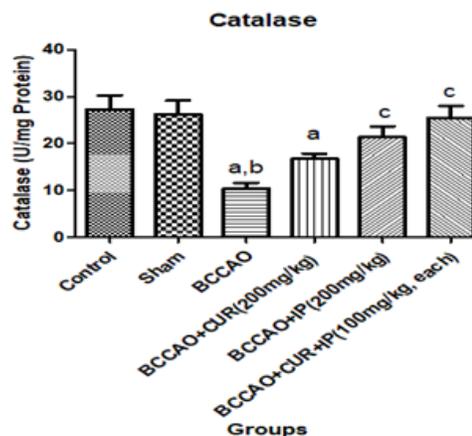


Figure 6: Values are expressed as mean ± SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison tests. Pa < 0.05 compared to Control, pb < 0.05 compared to sham, pc < 0.05 compared to BCCAO, respectively.

Effect of Experimental Groups on Tissue Reduced Glutathione (GSH)

On the basis of statistical analysis it was observed that the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg), Indian propolis (200 mg/kg), and curcumin (200 mg/kg) treated group showed statistical highly significant increase in the reduced glutathione (GSH) in the brain (44.51 ± 6.39 , $p < 0.001$), (39.26 ± 6.50 , $p < 0.01$), and (34.33 ± 4.11 , $p < 0.05$) as compared to BCCAO model (11.71 ± 1.75 , $p < 0.01$). BCCAO treated rats showed highly significant decrease in the GSH (6.33 ± 0.595 , $p < 0.001$) as compared with control (0.64 ± 0.28 , $p < 0.001$) and sham treated group (0.663 ± 0.191 , $p < 0.001$) as shown in Figure 5 and Table 2.

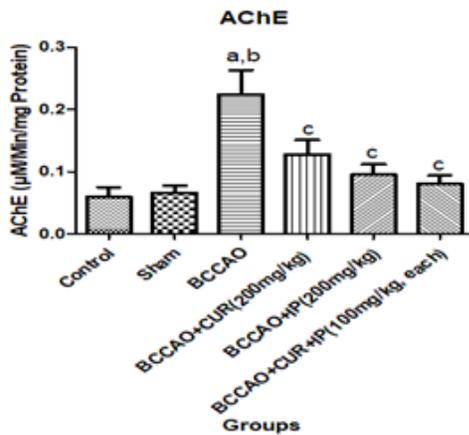


Figure 7: Values are expressed as mean \pm SEM (n=6). BCCAO=Bilateral common carotid artery occlusion, CUR=Curcumin, IP=Indian propolis. Values are statistically significant at $p < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparison tests. Pa < 0.05 compared to Control, pb < 0.05 compared to sham, pc < 0.05 compared to BCCAO, respectively.

Effect of Experimental Groups on Tissue Catalase (CAT)

On the basis of statistical analysis it was observed that the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) and Indian propolis (200 mg/kg) treated group showed statistical significant increase in the catalase (CAT) in the brain (25.40 ± 2.63 , $p < 0.01$) and (21.35 ± 2.30 , $p < 0.05$) as compared to BCCAO model (10.34 ± 1.26 , $p < 0.001$). BCCAO and curcumin (16.73 ± 1.13 , $p < 0.001$) treated rats showed highly significant decrease in the catalase as compared with control (28.27 ± 2.17 , $p < 0.001$) and sham treated group (26.18 ± 3.01 , $p < 0.001$) as shown in Figure 6 and Table 2.

Effect of Experimental Groups on Tissue Acetylcholine Esterase (AChE)

On the basis of statistical analysis it was observed that the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg), Indian propolis (200 mg/kg) and curcumin (200 mg/kg) treated group showed statistical significant decrease in the acetylcholinesterase (AChE) in the brain (0.080 ± 0.013 , $p < 0.001$), (0.095 ± 0.17 , $p < 0.001$) and (0.128 ± 0.023 , $p < 0.01$) as compared to BCCAO model (0.224 ± 0.039 , $p < 0.001$). BCCAO treated rats showed highly significant increase in the AChE as compared with control (0.060 ± 0.015 , $p < 0.001$) and sham treated group (0.069 ± 0.007 , $p < 0.001$) as shown in Figure 7 and Table 2.

Determination of H and E on Experimental Groups

In the present study on rats it was found that tissues were properly arranged without any pathological changes in control and sham group as depicted in slide a and b, respectively. There were major inflammation; edema, glial reaction, karyomegaly, and necrosis were observed in BCCAO group as depicted in slide c. Well preserved parenchyma with minimal inflammation in the curcumin (200 mg/kg) treated group and minimal glial reaction and minor edema was observed in the Indian propolis (200 mg/kg) treated group as depicted in slide

d and e, respectively. While the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) showed improvement in the histological properties is better than the curcumin (200 mg/kg) and Indian propolis (200 mg/kg) treated individually because there was no significant pathology and the parenchyma was well preserved depicted in slide f. All slides are depicted in Figure 8.

DISCUSSION

The results of this study showed that occlusion of the bilateral common carotid artery produced neurological deficits, biochemical and histological alteration in rats. However, the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) improved the neurological deficits and also reduced oxidative stress as indicated by increases in endogenous antioxidant molecules (GSH and catalase) and decreased MDA levels in the brains of rats subjected to BCCAO. The combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) also inhibited the AChE activity in the brain of rats. BCCAO is a well characterized and classical model for the induction of VaD.³⁸ Moreover, it was found to decrease brain antioxidant defense systems such as GSH and CAT levels and the increased levels of MDA, suggesting increased lipid peroxidation due to oxidative stress.³⁹ Additionally, the cholinergic system has a very important role in cognitive processes and based on the studies exploring cholinergic changes in the experimental models of VaD, it has been documented that cholinergic deficits with increased AChE activity is closely related to the pathophysiology of cognitive impairments.⁴⁰ Thus, the brain activity of AChE, a major biomarker of cholinergic function was measured in this study. Our data showed that animals subjected to BCCAO model, had increased brain activity of AChE, the enzyme responsible for the degradation of ACh. Previous studies have established that a decreased synaptic concentration of ACh plays a crucial role in cognitive dysfunctions in stroke.⁴¹ Thus, the ability of the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) to reduce brain activity of AChE in BCCAO rats suggests that it might enhance cognitive function in the VaD rats. Meanwhile, it is important to note that Indian propolis has been reported to contain the important biologically active compounds namely caffeic acid phenethyl ester, pinocembrin, and galangin. These compounds have been reported to have neuroprotective effect.⁴² Curcumin, another magical herb explored for its diverse pharmacological activity exhibit the neuroprotective property also.⁴³ In the study it was found that the combination of the Indian propolis with curcumin synergizes the effect of each other to prevent the progression of VaD.

CONCLUSION

In the present study, it was concluded that the observed cognitive impairment and neurodegeneration are associated with increased oxidative stress, declined cholinergic neuro-

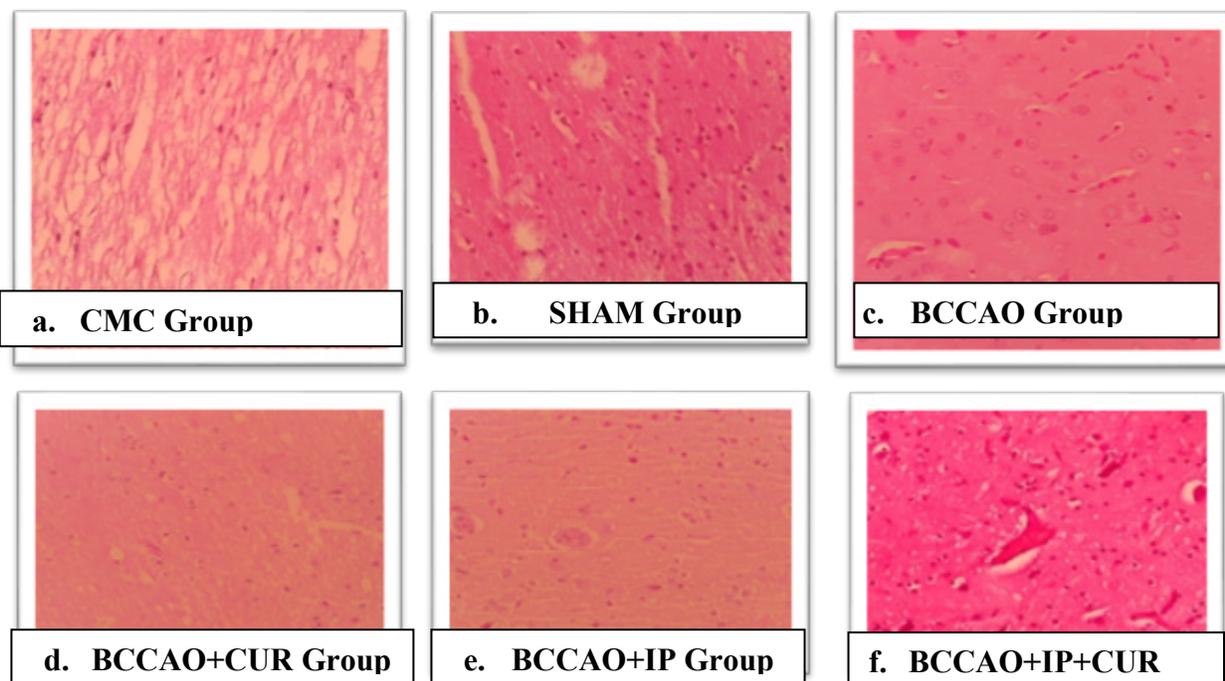


Figure 8: Histopathological images of rat brain from different treatment groups, where an individual image represents the brain for the following treatment groups in the respective slides as: (a) Group I- Control model group (CMC), (b) Group II- Sham operated group (SHAM), (c) Group III- Bilateral common carotid artery occlusion group (BCCAO), (d) Group IV-Curcumin group (BCCAO+CUR 200 mg/kg), (e) Group V- Indian propolis group (BCCAO+IP200 mg/kg), (f) Group VI- Combination of Indian propolis and curcumin (BCCAO+IP, 100 mg/kg+CUR,100 mg/kg)

transmission, and neurochemical alterations in the brain due to the induction of BCCAO. The result of present study elicits that the combination of Indian propolis (100 mg/kg) with curcumin (100 mg/kg) was much effective for neuroprotection than curcumin (200 mg/kg) and Indian propolis (200 mg/kg) individually. However Indian propolis individually also provided significant effect than individual curcumin which results that Indian propolis in combination with curcumin could be better interventions for the treatment of VaD, and the observed activities will be more beneficial and novel in grabbing the researcher's attention and emphasizing future research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

REFERENCES

- O'Brien JT, A omas. Vascular dementia, e Lancet. 2015;386(10004):1698-706.
- Jinu KV, Goothy SS, Karunakaran B. Dementia: A silent killer which kills millions in its wake: An overview.
- Uwagbai O, Kalish VB. Vascular dementia. InStatPearls [Internet] 2022 Jan 14. StatPearls Publishing.
- Yang GX, Sun JM, Zheng LL, Zhang L, Li J, Gan HX, Huang Y, Huang J, Diao XX, Tang Y, Wang R. Twin drug design, synthesis and evaluation of diosgenin derivatives as multitargeted agents for the treatment of vascular dementia. *Bioorganic & Medicinal Chemistry*. 2021 May 1;37:116109..
- Liang J, Huang YY, Zhou Q, Gao Y, Li Z, Wu D, Yu S, Guo L, Chen Z, Huang L, Liang SH. Discovery and optimization of α -mangostin derivatives as novel PDE4 inhibitors for the treatment of vascular dementia. *Journal of Medicinal Chemistry*. 2020 Mar 2;63(6):3370-80.
- Diken ME, YILMAZ B. Inhibitory effect on acetylcholinesterase and toxicity analysis of some medicinal plants. *International Journal of Secondary Metabolite*. 2022;9(1):27-42.
- Chen YD, Zhang J, Wang Y, Yuan JL, Hu WL. Efficacy of cholinesterase inhibitors in vascular dementia: an updated meta-analysis. *European neurology*. 2016;75(3-4):132-41.
- Battle CE, Abdul-Rahim AH, Shenkin SD, Hewitt J, Quinn TJ. Cholinesterase inhibitors for vascular dementia and other vascular cognitive impairments: a network meta-analysis. *Cochrane Dementia and Cognitive Improvement Group, editor. Cochrane Database Syst Rev [Internet]*. 2021 Feb 22 [cited 2022 Aug 23];2021(2). Available from: <http://doi.wiley.com/10.1002/14651858.CD013306.pub2>
- Xing S huai, Zhu C xiao, Zhang R, An L. Huperzine A in the Treatment of Alzheimer's Disease and Vascular Dementia: A Meta-Analysis. *Evid Based Complement Alternat Med*. 2014;2014:1-10.
- Subedi L, Gaire BP. Neuroprotective Effects of Curcumin in Cerebral Ischemia: Cellular and Molecular Mechanisms. *ACS Chem Neurosci*. 2021 Jul 21;12(14):2562-72.
- Zhang Y, Miao L, Lin L, Ren CY, Liu JX, Cui YM. Repeated administration of Sailuotong, a fixed combination of Panax ginseng, Ginkgo biloba, and Crocus sativus extracts for vascular dementia, alters CYP450 activities in rats. *Phytomedicine*. 2018 Jan;38:125-34.
- Kongkeaw C, Dilokthornsakul P, Thanarangarit P, Limpeanchob N, Norman Scholfield C. Meta-analysis of randomized controlled trials on cognitive effects of Bacopa monnieri extract. *J Ethnopharmacol*. 2014 Jan;151(1):528-35.

13. Chaudhary R, Singh R, Verma R, Kumar P, Kumar N, Singh L, et al. Investigation on protective effect of *Terminalia bellirica* (Roxb.) against drugs induced cardiotoxicity in wistar albino rats. *J Ethnopharmacol*. 2020 Oct;261:113080.
14. Pant K, Thakur M, Chopra HK, Nanda V, Javed Ansari M, Pietramellara G, et al. Characterization and discrimination of Indian propolis based on physico-chemical, techno-functional, thermal and textural properties: A multivariate approach. *J King Saud Univ - Sci*. 2021 Jun;33(4):101405.
15. Kapare H, Lohidasan S, Sinnathambi A, Mahadik K. Standardization, anti-carcinogenic potential and biosafety of Indian propolis. *J Ayurveda Integr Med*. 2019 Apr;10(2):81–7.
16. Kasote DM, Pawar MV, Gundu SS, Bhatia R, Nandre VS, Jagtap SD, et al. Chemical profiling, antioxidant, and antimicrobial activities of Indian stingless bees propolis samples. *J Apic Res*. 2019 Aug 8;58(4):617–25.
17. Sadhana N, Lohidasan S, Mahadik KR. Marker-based standardization and investigation of nutraceutical potential of Indian propolis. *J Integr Med*. 2017 Nov;15(6):483–94.
18. Ramnath S, Venkataramgowda S. Antioxidant Activity of Indian Propolis - An In Vitro Evaluation. *Int J Pharmacol Phytochem Ethnomedicine*. 2016 Oct 1;5:79–85.
19. Abbasi AJ, Mohammadi F, Bayat M, Gema SM, Ghadirian H, Seifi H, et al. Applications of Propolis in Dentistry: A Review. *Ethiop J Health Sci [Internet]*. 1970 Jan 1 [cited 2022 Aug 23];28(4). Available from: <https://www.ajol.info/index.php/ejhs/article/view/175557>
20. Gupta N, Verma K, Nalla S, Kulshreshtha A, Lall R, Prasad S. Free Radicals as a Double-Edged Sword: The Cancer Preventive and Therapeutic Roles of Curcumin. *Molecules*. 2020 Nov 18;25(22):5390.
21. Ren B, Zhang Y, Liu S, Cheng X, Yang X, Cui X, et al. Curcumin alleviates oxidative stress and inhibits apoptosis in diabetic cardiomyopathy via Sirt1-Foxo1 and PI3K-Akt signalling pathways. *J Cell Mol Med*. 2020 Nov;24(21):12355–67.
22. Targhi AA, Moammeri A, Jamshidifar E, Abbaspour K, Sadeghi S, Lamakani L, et al. Synergistic effect of curcumin-Cu and curcumin-Ag nanoparticle loaded niosome: Enhanced antibacterial and anti-biofilm activities. *Bioorganic Chem*. 2021 Oct;115:105116.
23. Gu Y, Zhu Y, Deng G, Liu S, Sun Y, Lv W. Curcumin analogue AI-44 alleviates MSU-induced gouty arthritis in mice via inhibiting cathepsin B-mediated NLRP3 inflammasome activation. *Int Immunopharmacol*. 2021 Apr;93:107375.
24. Khoshnam SE, Sarkaki A, Khorsandi L, Winlow W, Badavi M, Moghaddam HF, et al. Vanillic acid attenuates effects of transient bilateral common carotid occlusion and reperfusion in rats. *Biomed Pharmacother*. 2017 Dec;96:667–74.
25. Venkat P, Chopp M, Chen J. Models and mechanisms of vascular dementia. *Exp Neurol*. 2015 Oct;272:97–108.
26. Impellizzeri D, Siracusa R, Cordaro M, Crupi R, Peritore AF, Gugliandolo E, et al. N-Palmitoylethanolamine-oxazoline (PEA-OXA): A new therapeutic strategy to reduce neuroinflammation, oxidative stress associated to vascular dementia in an experimental model of repeated bilateral common carotid arteries occlusion. *Neurobiol Dis*. 2019 May;125:77–91.
27. Jiang T, Yu JT, Zhu XC, Zhang QQ, Tan MS, Cao L, et al. Ischemic Preconditioning Provides Neuroprotection by Induction of AMP-Activated Protein Kinase-Dependent Autophagy in a Rat Model of Ischemic Stroke. *Mol Neurobiol*. 2015 Feb;51(1):220–9.
28. Bowen MT, Peters ST, Absalom N, Chebib M, Neumann ID, McGregor IS. Oxytocin prevents ethanol actions at δ subunit-containing GABA_A receptors and attenuates ethanol-induced motor impairment in rats. *Proc Natl Acad Sci*. 2015 Mar 10;112(10):3104–9.
29. Wahul AB, Joshi PC, Kumar A, Chakravarty S. Transient global cerebral ischemia differentially affects cortex, striatum and hippocampus in Bilateral Common Carotid Arterial occlusion (BCCAO) mouse model. *J Chem Neuroanat*. 2018 Oct;92:1–15.
30. Kaundal M, Zameer S, Najmi AK, Parvez S, Akhtar M. Betulinic acid, a natural PDE inhibitor restores hippocampal cAMP/cGMP and BDNF, improve cerebral blood flow and recover memory deficits in permanent BCCAO induced vascular dementia in rats. *Eur J Pharmacol*. 2018 Aug;832:56–66.
31. Pujari RR, Vyawahare NS, Thakurdesai PA. Neuroprotective and antioxidant role of Phoenix dactylifera in permanent bilateral common carotid occlusion in rats. *J Acute Dis*. 2014;3(2):104–14.
32. Silva P, Nazrath N, Baliga M, D'silva P, Dsouza M, Arnadi R, et al. Lipid peroxidation, copper and vitamin C in lens and serum of people with diabetes and senile cataracts: A comparative evaluation. 2017 Aug 18;
33. Shivani C, Lubhan S, Kedarinath T, Vipul K. Neuroprotective effect of Silibinin against middle cerebral artery occlusion induced focal cerebral ischemia and brain injury in Wistar rats. *J Neurosci Behav Health*. 2017 Jul 31;9(1):10–5.
34. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. *Data Brief*. 2016 Mar;6:194–9.
35. Haider S, Tabassum S, Perveen T. Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: A comparative study. *Brain Res Bull*. 2016 Oct;127:234–47.
36. Schmatz R, Mazzanti CM, Spanevello R, Stefanello N, Gutierrez J, Corrêa M, et al. Resveratrol prevents memory deficits and the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats. *Eur J Pharmacol*. 2009 May;610(1–3):42–8.
37. Liu B, Gao JM, Li F, Gong QH, Shi JS. Gastrodin Attenuates Bilateral Common Carotid Artery Occlusion-Induced Cognitive Deficits via Regulating A β -Related Proteins and Reducing Autophagy and Apoptosis in Rats. *Front Pharmacol*. 2018 Apr 26;9:405.
38. Zheng Y, Zhang J, Zhao Y, Zhang Y, Zhang X, Guan J, et al. Curcumin protects against cognitive impairments in a rat model of chronic cerebral hypoperfusion combined with diabetes mellitus by suppressing neuroinflammation, apoptosis, and pyroptosis. *Int Immunopharmacol*. 2021 Apr;93:107422.
39. Umukoro S, Oghwere EE, Ben-Azu B, Owoeye O, Ajayi AM, Omorogbe O, et al. Jobelyn® ameliorates neurological deficits in rats with ischemic stroke through inhibition of release of pro-inflammatory cytokines and NF- κ B signaling pathway. *Pathophysiology*. 2019 Mar;26(1):77–88.
40. Fanoudi S, Alavi MS, Hosseini M, Sadeghnia HR. Nigella sativa and thymoquinone attenuate oxidative stress and cognitive impairment following cerebral hypoperfusion in rats. *Metab Brain Dis*. 2019 Aug;34(4):1001–10.
41. Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules*. 2020 Dec 8;25(24):5789.
42. Nanaware S, Shelar M, Sinnathambi A, Mahadik KR, Lohidasan S. Neuroprotective effect of Indian propolis in β -amyloid induced memory deficit: Impact on behavioral and biochemical parameters in rats. *Biomed Pharmacother*. 2017 Sep;93:543–53.
43. Subedi L, Gaire BP. Neuroprotective Effects of Curcumin in Cerebral Ischemia: Cellular and Molecular Mechanisms. *ACS Chem Neurosci*. 2021 Jul 21;12(14):2562–72.