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Original Article

NEUROPROTECTIVE EFFECTS OF CROMAKALIM ON CEREBRAL ISCHEMIA-REPERFUSION (IR) INJURY AND ALUMINIUM INDUCED TOXICITY IN RAT BRAIN

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ABSTRACT

Objective: Preclinical studies have demonstrated that potassium channel openers show a neuroprotective effect on cerebral ischemia-reperfusion (IR) injury in rats. However, their mechanism of action and effects in brain stroke related disorders such as Alzheimer's disease (AD) remain poorly understood. Hence present study was done to investigate the prophylactic use of the adenosine triphosphate-sensitive (ATP) potassium channel opener cromakalim on neurological function in rats with cerebral IR injury and its related disorders AD.

Methods: Male Wistar rats were randomly assigned to 6 groups (n = 36): Normal control, aluminium chloride($AlCl_3$) control, $AlCl_3$ Sham operated, ischemia reperfusion (IR) control, $AlCl_3$-IR control and $AlCl_3$-IR with cromakalim treatment. Cromakalim10 mg/kg intra peritonially (i.p.) was administered daily for 42 days in middle cerebral artery occluded rats treated with $AlCl_3$. At 24 hours post-surgery, neurological score, brain hemisphere weight difference, brain acetylcholinestarase level, lipid peroxidation, Na^+/K^+ ATPase pump activity and super oxide dismutase level were measured.

Results: Following cerebral ischemia-reperfusion injury along with aluminium chloride, neurological score,brain hemisphere weight difference, brain acetylcholinestarase level, malondialdehyde levels were significantly high while Na^+/K^+ ATPase pump activity and super oxide dismutase(SOD) levels were significantly lower in diseased animals ($P < 0.05$). Treatment with cromakalim significantly reduced neurological score, brain hemisphere weight difference, brain acetyl cholinestarase level, malondialdehyde levels while raised Na^+/K^+ ATPase pump activity and super oxide dismutase levels ($P < 0.05$).

Conclusion: Hence results of our study demonstrated the neuroprotective potential of cromakalimin cerebral IR injury and its related disorders AD.

Keywords: Cerebral ischemia-reperfusion, Cromakalim. Aluminium, Alzheimer's disease, Oxidative stress, Neuroprotection.

INTRODUCTION

Stroke is an interruption in the supply of blood to a particular part of the brain. There are two main causes of stroke. Either a blood vessel within the brain becomes clogged or blocked (ischemic stroke-IS, about 83% of strokes) or it ruptures, causing blood to leak into the brain (haemorrhagic stroke) [1].It is well reported that stroke more than doubles the risk of developing dementia and AD and around one in three people who have a stroke develop dementia [2].Clinical diagnosis of ADis associated with considerably increased risk of stroke development and patients with AD had a higher risk of IS (ischemic stroke) and ICH (intracerebral hemorrhage) [3]. Further epidemiological studies show that risk factors for AD have a vascular basis and practically all drugs reported to slow the development of AD, improve or increase cerebral perfusion. Evidence that cerebral hypoperfusion appears to precede the cognitive, and degenerative pathology that is present in AD. Current treatment of brain strokeincludes tissue plasminogen activators, antiplatelet agents and anticoagulants for their antithrombotic effects. Reports showed that free radical scavengers, minocycline and growth factors have shown neuroprotective effects in the treatment of stroke, while antihypertensive drugs, lipid-lowering drugs and oral antidiabetic drugs have shown beneficial effects for the prevention of stroke [4]. However, these agents are associated with number of side effects. Therefore, there is a need to identify new targets and molecules in central nervous system (CNS) that provide neuroprotection in stroke related disorders. One of them is the ion channel for Na^+, K^+, Ca^{2+} and Cl^- . They play a crucial role in controlling a very wide spectrum of physiological processes. Their dysfunction can produce various CNS diseases. Hence new generation of therapeutic agents are expected to result from targeting this ion channels.

Further, it is well established that K^+ channels are widely expressed in CNS and are gaining attention for their beneficial roles in neuroprotection. Dysfunction of potassium channel leads to change in neuronal physiological processes and results in pathophysiology of various diseases including stroke, epilepsy, pain and cognition [5].Hence K^+ channels are recognized as potential therapeutic targets in the treatment of CNS disorders. Adenosine triphosphate-sensitive potassium channel (K_{ATP}) openers such as cromakalim provide neuroprotection against cerebral ischemia-reperfusion injury by reducing inflammation and free radical mediated oxidative stress. Neuroprotective effects of cromakalim have been already established on cerebralischemia-reperfusion injury in rats [6].Hence present study hypothesized that K_{ATP} openers couldreduce strokeas well as stroke related disorderssuch as AD by providing neuroprotection. Rat model of cerebral ischemia-reperfusion was established through middle cerebral artery occlusion using the suture method [6] and this was preceded by $AlCl_3$ to induce cholinergic nerve damage as that of Alzheimer's disease in same animals [7]. The effects of prophylactic useof the K_{ATP} channel opener, cromakalim, on neurologicalfunction, brain acetylcholinestares level and lipid peroxidation parameters were determined to explore the mechanisms of action of cromakalimfor neuroprotection.

MATERIALS AND METHOD

Drugs and Chemicals

Cromakalim was gifted from Dr. Anjali Tarai, Assistant Professor, Department of Pharmacology, VSS Medical College, Cuttack, Orissa, India. Other chemicals used in experiment were of laboratory grade. Cromakalim solution was prepared freshly by dissolving it in sterile distilled water and injected i.p. with dose 10 mg/kg. $AlCl_3$ solution was made freshly in sterile distilled water at the beginning of each experiment and given orally at dose of 100 mg/kg.

Animals

Healthy adult male Wistar rats (250 gm) aged 8 months were selected for the study. Animals were maintained at 18± 2°C and kept in well ventilated animal house in polypropylene cages with free

access to food and water. All studies was done with prior permission from IAEC of Parul Institute of Pharmacy (921/PO/AC/05/ CPCSEA) and Study was approved as Protocol No PIPH 21/13.Animals were divided into six groups. Group 1 served as normal control (n=6) and received distilled water. Group 2 serve as $AlCl_3$ control and received $AlCl_3$at dose 100 mg/kg per oral for a period of 42 days (6 weeks).Group 3 served as $AlCl_3$ Sham operated (n=12) and treated with $AlCl_3$ at dose 100 mg/kg per oral for a period of 42 days (6 weeks).Sham operation was performed on 41th day. Group 4 served as ischemia reperfusion (IR) control (n=6) and received distilled water for 42 days. Cerebral ischemia –reperfusion was performed on 41th day from initiation of experiment. Group 5 served as $AlCl_3$-IR control (n=6) and animals were treated with $AlCl_3$ at dose 100 mg/kg per oral for a period of 42 days (6 weeks). Cerebral ischemia –reperfusion was performed on 41th day from initiation of experiment. Group 6 treated with$AlCl_3$and cromakalim (n=6) along with IR injury. These animals were treated with $AlCl_3$ at dose 100 mg/kg per oral and cromakalim at dose 10 mg/kg (i.p.) for a period of 42 days (6 weeks). Cerebral IR was performed on 41th day from initiation of experiment. Cerebral IR was induced as per MCAO (transient middle cerebral artery occlusion) method [6]. Rats were anesthetized with an i.p. injection of 100 mg/kg ketamine. A 2–3 cm incision was made in the middle of the neck line, separating the left carotid artery, the superior thyroid artery, and the occipital artery, as well as the internal and external carotid communicating arteries. The occipital artery branches of external carotid artery (ECA) were isolated and tied with a cotton thread.Cotton thread was tied loosely around the ECA stump near the bifurcation. Then internal carotid artery (ICA) and common carotid artery (CCA) were temporarily occluded by fine vessel clip.

Through a small incision to the ECA stump, blunt Poly-L-lysine coated 4-0 monofilament was inserted from the left external carotid artery into the left internal carotid artery to a depth of 18.0mm, vessel clip from ICA removed. After a variable length of suture had been inserted into ECA stump, resistance was felt and slight curving of suture was observed, indicating that suture had passed middle cerebral artery (MCA) origin and reached to proximal segment of anterior cerebral artery (ACA-it has small diameter).Hence suture had blocked all sources of blood from ICA, ACA and posterior cerebral artery. Finally vessel clip from CCA was removed to restore blood flow. The midline incision was closed, leaving suture protruding so it could be withdrawn to allow reperfusion.The thread was maintained for 2 hours and subsequently removed to restore blood flow to the common carotid and internal carotid arteries.Here 18 mm of suture was pulled back until resistance was felt, indicating that tip cleared the ACA-ICA lumen and was in ECA stump, then trimmed. The animals were transfer to a fresh cage with free access to food and water.

Tissue homogenate preparation

At the end of the study, animals were sacrificed, brain were dissected out and washed with 0.9% NaCl and stored at –40 °C for further processing. Brain was homogenized in 10% (w/v) ice-cold 0.1 M phosphate buffer solution (pH 7.4). The lipid peroxidation (LPO) was estimated with a part of crude homogenate and the rest homogenate was centrifuged at 12,000 rpm for 20 min to obtain the supernatant (S) that was used for enzymatic estimations [7].

Tissue total protein level

It was measured using bovine serum albumin as standard, alkaline copper reagent Solution A (2% sodium carbonate in 0.1 N NaOH solution in distilled water) and solution B(0.5% copper sulphate in 1.0% sodium potassium tartarate).50.0 ml of solution A was mixed with 1.0ml of solution B just before use. Folin's phenol reagent was obtained commercially. One volume of Folin's phenol reagent was diluted with 1 volume of distilled water just before use. 20.00 mg standard bovine serum albumin (BSA) was dissolved in 100 ml distilled water. Few drops of NaOH were added to aid complete dissolution of BSA and to avoid frothing. 10ml of the stock was diluted to 100ml to get a working standard (200 µg/ml). Procedure 0.1ml crude homogenate was used for protein extraction. The protein was extracted by mixing with 5% cold trichloroacetic acid and centrifuged. The pellet was solubilized with 0.5 N sodium hydroxide and stored over-night at room temperature. After neutralization with 0.5 N HCl, 0.2 ml of diluted solution and different concentrations of standard were taken. The volume was made up to 1.0 ml with distilled water. Blank contained 1.0 ml distilled water.

To all the tubes 5.0 ml alkaline copper reagent was added and left at room temperature for 10 Min. 0.5 ml of folin's phenol reagent was added and the blue colour developed was read after 20 minutes at 660nm against reagent blank in a spectrophotometer. Protein concentration is expressed as mg/gm of wet brain tissue [8].

Brain acetylcholinestarase level

It is a marker of the loss of cholinergic neurons in the forebrain. The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30 s intervals at 412 nm. Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed/ min/mg of protein [9].

Malondialdehyde (MDA)

It is a marker of lipid peroxidation. 1 ml of homogenate was incubated at 37 °C for 10 min. 1 ml of 10% trichloroacetic acid (TCA) chilled (w/v) was added to it and centrifuged at 2500 rpm for 15 min at room temperature. 1 ml of 0.67% TBA was added to 1 ml of supernatant and kept in a boiling water bath for 10–15 min. The tubes were cooled under tap water. After cooling 1 ml of distilled water was added to it and absorbance was taken at 530 nm. The results were expressed as micromoles/mg of protein [10].

Superoxide dismutase (SOD)

An aliquot of 0.25 ml ice-cold chloroform was added to 0.1 ml of supernatant followed by addition of 0.15 ml ice-cold ethanol. The mixture was centrifuged at 3000 rpm for 10 min at 4 °C. 0.2 ml the supernatant was taken and 1.3 ml buffer, 0.5 ml EDTA, and 0.8 ml water were added. Reaction was started by adding 0.2 ml epinephrine. Change in absorbance ΔOD (optical density)/min at 480 nm was read for 3 min. The results were expressed in terms of munits/mg protein.One unit of enzyme activity is defined as the concentration required for the inhibition of the chromogen production by 50 % in one minute under the defined assay conditions [11].

Na^+/ K^+ ATPase pump activity

The incubation mixture containing 0.1 ml of each i.e tris HCL , 50 mM magnesim sulphate , 650 mM NaCl , 50 mM KCl , 1mM EDTA , 40 mM ATP and brain homogenate . Mixture was incubated at 36 ˚C for 15 minute. The reaction was arrested by addition of 1ml 10 % TCA and resultant mixture was centrifuged for 5 min.0.5 ml supernatant was taken, 2.5 ml,0.5 ml of 2.5 % ammonium molybdate and 0.25 ml ANSA (1-amino 2-napthol 4-sulphonic acid)was added.The mixture was incubated at 37 ˚C for 10 minutes. The intensity of blue color developed against blank at 620 nm. Results were expressed as nmoles of inorganic phosphorus liberated/min/mg protein. Standard calibration curve was prepared using potassium dihydrogen phosphate (2-15 µg/ml) [12].

Brain hemisphere weight difference

The MCA occluded animals were anaesthetised with high dose of pentobarbitone. The skull was opened and whole brain was removed immediately, rinsed with ice cold distilled water followed by 20 % sucrose and dried using bloating paper. The ipsilateral and contralateral parts of brain are isolated and weighed on digital balance. Weight difference was calculated between ipsilateral and contralateral brain region of each animal [13].

Neurobehavioral function scores

Neurological score was observed after 24 hours of IR[14].This score was observed for group 4, 5 and 6.

Score 0: No apparent deficit

Score 1: Contralateral forelimb flexion when suspended by tail

Score 2: Decreased grip of the contralateral forelimb while tail pulled

Score 3: Spontaneous movement in all direction or contralateral circling only if pulled by tail.

Score 4: Spontaneous contralateral circling

Score 5: Death after recovery from anaesthesia.

Statistical methods

Data are expressed as mean values ± S.E.M and analyzed using one-way ANOVA followed by Post-hoc Tukey's tests to identify two group differences using Prism software at 5% significance level.

RESULTS

Brain acetylcholinesterase levels

Administration aluminium chloride for 42 days significantly raised cholinesterase level in $AlCl_3$ treated control animals (73.07 ± 2.05) and $AlCl_3$ Sham operated animals (64.10 ± 0.84) when compared with normal animals(19.49 ± 0.55).Ischemia reperfusion(IR) injury along with $AlCl_3$also significantly raised (96.86 ± 1.07) cholinesterase level when compared with IR control (24.01 ± 0.54).

Treatment of cromakalim significantly reduced brain actetylcholinesterase level (26.33 ± 0.37) when compared with AlCl3 IR control animals (table 1).

Brain malondialdehyde(MDA) levels(n=6)

Administration aluminium chloride for 42 days significantly raised MDA level in $AlCl_3$ treated control animals (128.92 ± 3.46) and $AlCl_3$ Sham operated animals (78.72 ± 1.46) when compared with normal animals(14.20 ± 1.86).Ischemia reperfusion(IR) injury along with $AlCl_3$ also significantly raised (121.143 ± 1.67) MDA level when compared with IR control (77.23 ± 1.94). Treatment of cromakalim significantly reduced brain MDA level (39.90 ± 0.45) when compared with $AlCl_3$ IR control animals (table 2).

Brain SOD levels (n=6)

Administration aluminium chloride for 42 days significantly reduced SOD level in $AlCl_3$ treated control animals (84.54 ± 0.83) and $AlCl_3$ Sham operated animals (73.59 ± 0.85) when compared with normal animals(259.76 ± 0.80).Ischemia reperfusion(IR) injury along with $AlCl_3$ also significantly reduced (16.33 ± 1.93) SOD level when compared with IR control (22.78 ± 0.48).

Treatment ofcromakalim significantly raised brain SODlevel (143.49 ± 0.68) when compared with $AlCl_3$ IR control animals (table 3).

Na^+/K^+ ATPase activity (nm/mg protein) (n=6)

Administration aluminium chloride for 42 days significantly reduced Na^+/K^+ ATPase activity level in $AlCl_3$ treated control animals (37.24 ± 0.60) and $AlCl_3$ Sham operated animals (22.12 ± 0.39) when compared with normal animals(61.27 ± 0.47).

Ischemia reperfusion (IR) injury along with $AlCl_3$ also significantly reduced (22.95 ± 0.43) Na^+/K^+ ATPase activity when compared with IR control (10.12 ± 0.38). Treatment of cromakalim significantly raised brain Na^+/K^+ ATP ase activity (47.96 ± 0.29) when compared with $AlCl_3$ IR control animals (table 4).

Table 1: Brain cholinesterase activity µ mol of acetyl thiocholine iodide hydrolyzed/ minute (min)/milligram (mg) of protein)(n=6)

Groups	Brain cholinesterase levels
Normal Control	19.49 ± 0.55
$AlCl_3$ control	73.07 ± 2.05*
$AlCl_3$ Sham operated	64.10 ± 0.84*
IR control	24.01 ± 0.54*
$AlCl_3$ IR control	96.86 ± 1.07**
$AlCl_3$cromakalim IR	26.33 ± 0.37***

* Significant different than normal control ($p<0.05$) , **Significant different than IR control group($p<0.05$) , *** Significant different than $AlCl_3$ IR control ($p<0.05$)

Table 2: Brain malondialdehyde (MDA) levels

Groups	MDA(µmoles/mg protein)
Normal Control	14.20 ± 1.86
$AlCl_3$ control	128.92 ± 3.46*
$AlCl_3$ Sham operated	78.72 ± 1.46*
IR control	77.23 ± 1.94*
$AlCl_3$ IR control	121.143 ± 1.67**
$AlCl_3$cromakalim IR	39.90 ± 0.45***

* Significant different than normal control ($p<0.05$) , **Significant different than IR control group ($p<0.05$) , ***Significant different than $AlCl_3$ IR control ($p<0.05$

Table 3: Brain SOD levels

Groups	SOD(munits/mg protein)
Normal Control	259.76 ± 0.80
$AlCl_3$ control	84.54 ± 0.83*
$AlCl_3$ Sham operated	73.59 ± 0.85*
IR control	22.78 ± 0.48*
$AlCl_3$ IR control	16.33 ± 1.93**
$AlCl_3$cromakalim IR	143.49 ± 0.68***

* Significant different than normal control ($p<0.05$) , ** Significant different than IR control group ($p<0.05$) , *** Significant different than $AlCl_3$ IR control ($p<0.05$)

Hemisphere weight difference (n=6)

Administration aluminium chloride for 42 days significantly raised brain hemisphere weight difference in $AlCl_3$ treated control animals (0.016 ± 0.002) and $AlCl_3$ Sham operated animals (0.022 ± 0.003) when compared with normal animals(0.0062 ± 0.0003).Ischemia reperfusion(IR) injury along with $AlCl_3$ also significantly increased (0.077 ± 0.003) brain hemisphere weight differencewhen compared with normal control. However, this is nonsignificant when compared with IR control (0.071. ± 0.003).

Treatment of cromakalim significantly reducedbrain hemisphere weight difference (0.039 ± 0.0038) when compared with $AlCl_3$ IR control animals (table 5).

Neurological score (n=6)

Treatment ofcromakalim significantly reduced brain neurological damage (1.2 ± 0.12) when compared with animals treated with IR along with $AlCl_3$ (4.22 ± 0.15) as well as IR control animals (3.20 ± 0.16) (table 5).

Table 4: Na^+/K^+ ATPase activity (nm/mg protein)

Groups	(nm/mg protein)
Normal Control	61.27 ± 0.47
$AlCl_3$ control	37.24 ± 0.60*
$AlCl_3$ Sham operated	22.12 ± 0.39*
IR control	10.12 ± 0.38*
$AlCl_3$ IR control	22.95 ± 0.43**
$AlCl_3$ cromakalim IR	47.96 ± 0.29***

* Significant different than normal control ($p<0.05$) , ** Significant different than IR control group ($p<0.05$) , *** Significant different than $AlCl_3$ IR control ($p<0.05$)

Table 5: Hemisphere weight difference

Groups	Gm/100 gm body weight
Normal Control	0.0062 ± 0.0003
$AlCl_3$ control	0.016 ± 0.002*
$AlCl_3$ Sham operated	0.022 ± 0.003*
IR control	0.071 ± 0.003*
$AlCl_3$ IR control	0.077 ± 0.003 (NS)
$AlCl_3$ cromakalim IR	0.039 ± 0.0038***

* Significant different than normal control ($p<0.05$) , *** Significant different than $AlCl_3$ IR control ($p<0.05$) , NS is Non-significant when compare with IR control

Table 6: Neurological score

Groups	Gm/100 gm body weight
IR control	3.20 ± 0.16
$AlCl_3$ IR control	4.22 ± 0.15
$AlCl_3$cromakalim IR	1.2 ± 0.12*

* Significant different than $AlCl_3$ IR control ($p<0.05$)

DISCUSSION

In present study we have made an attempt to evaluate efficacy of K_{ATP} channel opener cromakalim against ischemic-reperfusion type of brain stroke model in $AlCl_3$treated rats. The symptoms produced by IR along with $AlCl_3$ resembles to a great extent with that observed in clinical status. Research studies have demonstrated that three months after ischemic stroke, about 30-40 % of people show signs of dementia, including impairments in learning, attention as well as ability to remember. This vascular dementia mainly results from the interruption of blood supply to brain during stroke. Hence to produce such clinical condition, we treated rats with $AlCl_3$ for 42 days. It has been suggested that aluminium is a contributing factor in the pathogenesis of Alzheimer's disease. Hence to induce neurotoxicity as observed in AD, aluminium chloride was used [7] and cerebral ischemia –reperfusion was performed on 41th day from initiation of experiment in same animals.Our objective of study was to investigate neuroprotective effect of cromakalim (potassium channel opener) on cerebral ischemic stroke in aluminum chloride induced neuronal toxicity in rats. Cromakalim is ATP sensitive potassium channel opener and it improves neurological function and reduced cerebral infarction volume in rats [6].

Studies have shown that oxidative stress is increased during acute cerebral ischemia and plays a key role in ischemic nerve injury. It is well reported that during IR injury along with aluminium anti-oxidant mechanisms are compromised and hence it raise lipid peroxidation (malondialdehyde-MDA level) and reduced super oxide dismutase (SOD) levels in diseased control animal [15].In present study, cromakalim significantly lowers value of MDA and increased level of SOD in IR-aluminium treated rats. Thus prevent lipid peroxidation and oxidative stress.This suggest anti-oxidant role of cromakalim to produce neuroprotection. Aluminium crosses the blood brain barrier and induces inflammatory responses and inhibits long-term potentiation, and causes synaptic structural abnormalities, thereby resulting in profound memory loss [16]. Aluminium raises brain acetylcholinesterase level, cause neurodegeneration and neuroinflammation.It itself results into oxidative damage to brain and cognitive dysfunction.

Aluminium has a biphasic effect on acetylcholinesterase activity, withan initial increase in the activity of this enzyme during the first 2 weeks of exposure followed by a markeddecrease. These results into slow accumulation of aluminium in the brain [17, 18, 19] and thiswould contribute to the increase in acetylcholinesterase activity as observed in the aluminium chloride treated rats.Further, it is well established that more level of same enzyme is responsible for Alzheimer'sdisease to damage cholinergic neurones. Chronic administration of cromakalim was found to reduce brain acetylcholinestarse level and also reduced oxidative damage induced by ischemia –reperfusion injury along with chronic aluminium administration. Hence from data of our study, cromakalim

treatment prevents cholinotoxic effect of aluminium on neurones in IR-aluminium treated rats. This is further supported by significant lower value of neurological damage score in cromakalim treated animals when compared with IR control animals and $AlCl_3$-IR animals.Cromakalim significantly raised Na^+/K^+ ATPase pump activity and reduced brain hemisphere weight difference. As it is well understood that neuronal cell damage by ischemic injury and by chronic aluminium administration interrupt activity of Na^+/K^+ ATPase pumps which maintain cellular water level. Due to inhibition of this pump, extracellular potassium accumulates and at the same time that sodium and water are sequestered intracellular and leading to cell swelling, its lysis and raised brain hemisphere weight difference [20].Hence our report show role of cromakalim in prevention of this cell injury.

Hence our results show that cromakalim significantly improves neurological functions at 24 hours post-surgery, and provide neuroprotection as observed by reduced acetylcholinestarase level, malondialdehyde level, the neurological score, brain hemisphere weight difference and by raising SOD level along with Na^+/K^+ pump activity when compared with disease control groups. These results suggest that prophylactic use of cromakalim could improve neurological functional and protect neurons as well as stroke related disorders in a rat model of cerebral ischemia-reperfusion injury with $AlCl_3$ induced neurotoxicity.

CONCLUSION

In present study, results suggests that pre-treatment with cromakalim (10mg/kg i.p.) produce neuroprotective effect in $AlCl_3$-IR animals as indicted by reduction in, neurological deficit, malondialdehyde, cholinesterase enzyme and raised anti-oxidant enzyme defence. This protective effect might be due to free radical scavenging effect, enhancing anti-oxidant enzyme and reducing cholinesterase activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Goldstein LB, Adams R, Alberts MJ, Appel LJ, Brass LM, Bushnell CD, et al. Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation 2006;113(24):e873-923.
2. Torre JC. Alzheimer Disease as Vascular Disorder-Nosological Evidence. Stroke 2002;33:1152-62.
3. Chi N-F, Chien L-N, Ku H-L, Hu C-J, Chiou H-Y. Alzheimer disease and risk of stroke: a population-based cohort study. Neurology 2013;80(8):705-11.
4. Kikuchi K, Uchikado H, Morioka M, Murai Y, Tanaka E. Clinical neuroprotective drugs for treatment and prevention of stroke. International journal of molecular sciences 2012;13(6):7739-61.
5. Alan GC, Susan EB, John AM. Therapeutic potential for potassium channel modulator for CNS disorders. Expert Opin Ther Pat 2003;13(1):23-32.
6. Wang S, Liu J, Chang Q, Li Y, Jiang Y. Neuroprotective effects of cromakalim on cerebral ischemia-reperfusion injury in rats. Neural Regen Res 2010;5(9):678-82.
7. Kumar A, Prakash A, Dogra S. Neuroprotective effect of carvedilol against aluminium induced toxicity: possible behavioral and biochemical alterations in rats. Pharmacological reports : PR 2011;63(4):915-23.
8. Lowry OH, Rosenbrough WJ, Farren AI, Randel RJ, J. Protein measurement with folin phenol reagent. Reagent 1951;93:265-75.
9. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358
11. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of biological chemistry 1972;247(10):3170-5.
12. Sovoboda P, Mossinger B. Catecholamaines and brain microsomal Na+ -K+ ATPase protection against lipoper oxidative damage. Biochem Pharmacol 1981;30:427-32.
13. Ahmad S. Angiotensin receptor antagonists delay nitric oxide-deficient stroke in stroke-prone rats. European journal of pharmacology 1997;333(1):39-45.
14. Menzies SA, Hoff JT, Betz AL. Middle cerebral artery occlusion in rats: A neurological and pathological evaluation of reproducible model. Neurosurg 2009;31:100-6.
15. Trigunayat A. Effect of ehanolic extract of H. perforatum on oxidative stress induced by cerebral ischemiareperfusion in rats Annal Neurosci 2009;16(1):6-9.
16. Roskams AJ, Connor JR. Aluminum access to the brain: a role for transferrin and its receptor. Proceedings of the National Academy of Sciences of the United States of America 1990;87(22):9024-7.
17. Kumar S. Biphasic effect of aluminium on cholinergic enzyme of rat brain. Neuroscience letters 1998;248(2):121-3.
18. Sharma P, Singh R. Dichlorvos and lindane induced oxidative stress in rat brain: Protective effects of ginger. Pharmacognosy research 2012;4(1):27-32.
19. Kawahara M, Muramoto K, Kobayashi K, Mori H, Kuroda Y. Aluminum promotes the aggregation of Alzheimer's amyloid beta-protein in vitro. Biochemical and biophysical research communications 1994;198(2):531-5.
20. Ramnathan N, Babu C, Justin A, Santhakumari S, J. Elucidation of neuroprotective role of endogenous GABA and energy metabolites in middle cerebral artery occluded model in rats. Ind Biol 2012;50:391-7.