ROLE OF GSK-3B IN REMOTE LIMB ISCHEMIC POST CONDITIONING AGAINST CEREBRAL ISCHEMIC REPERFUSION INJURY

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Introduction

- Restoration of blood flow with effective oxygenation to an ischemic organ, often paradoxically results in cerebral ischemic stroke, which is the second leading cause of death and acquired adult disability worldwide.
- In recent past, extensive exploitation of endogenous mechanism have led to development of effective cerebro protective treatment named ischemic pre/post conditioning (IPC/IPOC)
- In addition, the RIPC of femoral artery has been reported to be neuroprotective with better clinical efficacy against certain cerebral disorders possibly by alteration of various secondary messengers including RISK pathway, MAPK and ERK, NMDA, HDAC, GSK-3β and mPTP.
- Recent studies over past decade have highlighted the role of GSK-3β and mPTP modulation in cerebral stroke. However, the molecular mechanism for neuroprotection by RIPOC is yet to be explored.
- In the present study, we investigated the involvement of GSK-3β and mPTP in the neuroprotective role of RIPOC against cerebral stroke.

Materials and methods

Animals

Male Wistar rats were approved by Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC / RES / 17 / 07).

Experimental Design

Group1: Sham Control; Group 2: For ischemia reperfusion group (I/R), rats were subjected to 60 min of ischemia followed by 24 hours reperfusion; Group 3: In RIPOC group, after femoral artery I/R, rats were exposed to I/R injury by MCAO. The duration of occlusion and reperfusion of femoral artery was for 60 sec and repeated for four cycles; Group 4: GSK-3β inhibitor, Indirubin -3-monoxime (IM, 0.2 mg/kg, i.p.) was administered before reperfusion phase of RIPOC. Group 5: mPTP opener, carboxyatractyloside (CAT, 1mg/kg, i.p.) was administered before reperfusion phase of RIPOC.

Behavioral Parameters

Neurological Scoring (NSS): 18-point NSS was performed [1]. Rotarod Performance: Motor abnormalities were measured by latency to fall [2].

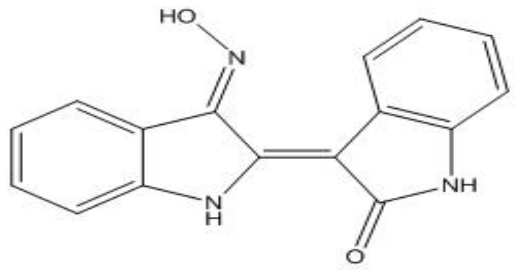
Biochemical Estimation

Oxidative stress marker, malondialdehyde (MDA) were quantitatively estimated as described by Wills [3]. Endogenous anti-oxidant like reduced glutathione (GSH) and catalase were estimated Ellman [4] and Sinha [5] respectively. TNF-α and GSK-3β estimation was performed according to the ELISA kit manufacturer's instructions.

Histological Examination

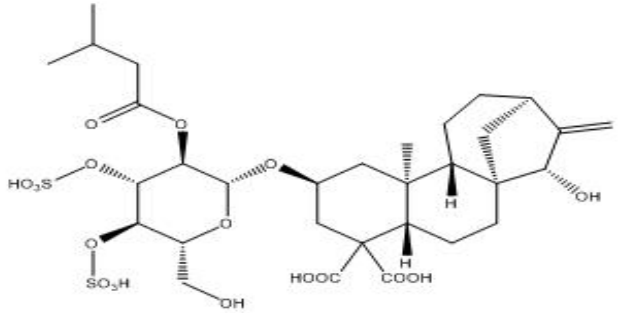
Hippocampal DG region has been stained with haematoxylin eosin stain and studied under bright field illumination using "Optika TCB5" microscope [6].

Hypothesis



Indirubin-3'-monoxime

GSK3β Inhibitor



Carboxyatractyloside

mPTP Opener

Synergistic neuroprotection with RIPOC

- Improved neurological Scoring
- More time spent on rotarod
- Attenuation of oxidative marker levels
- Amelioration of anti-oxidant levels
- Reduction in pro-inflammatory cytokines
- High healthy neuronal count
- Enhanced GSK-3β activity

Abolished neuroprotective role of RIPOC

- Visible neurological deficits
- Latency time on rotarod will decrease
- Significant rise of oxido-nitrative stress
- Weakened anti-oxidant system
- Increased neuroinflammation
- Substantial pyknotic neuronal count
- Low activity of GSK-3β

Results

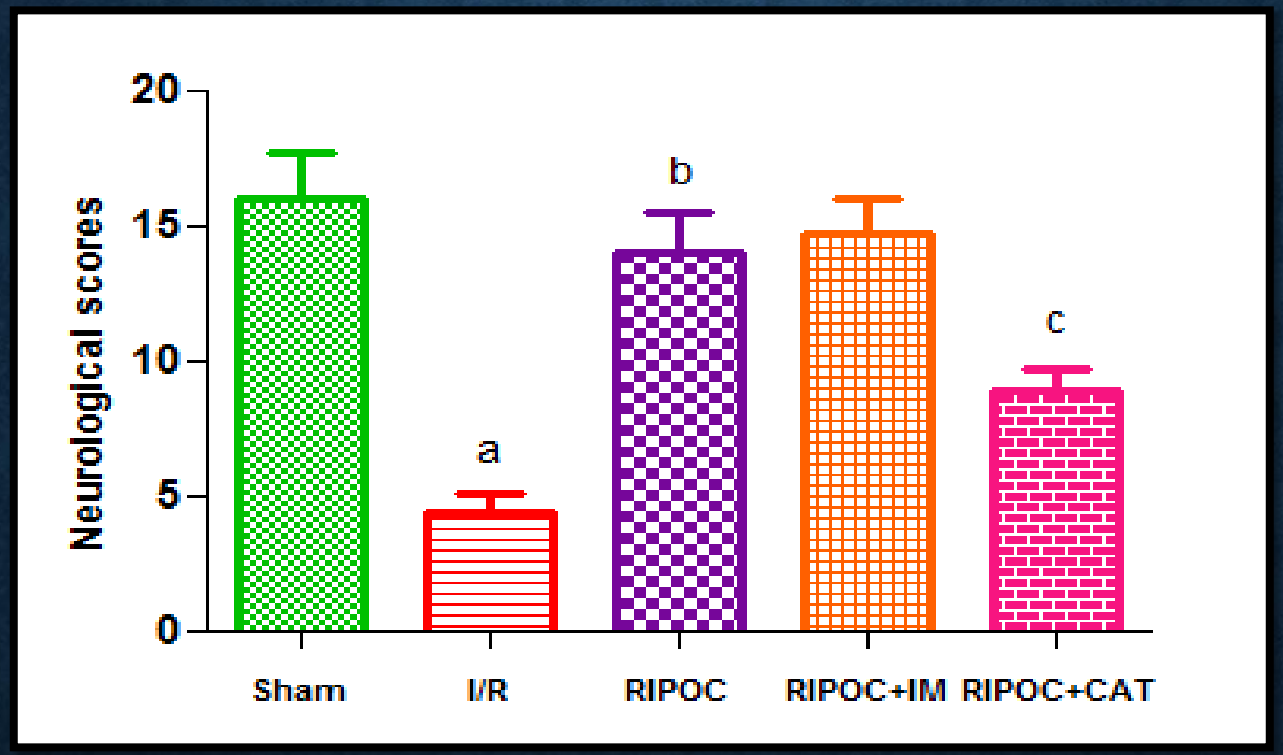


Fig.2. Effect of RIPOC, IM and CAT on neurological scoring.
[a]P<0.001 vs sham, [b]P<0.001 vs I/R

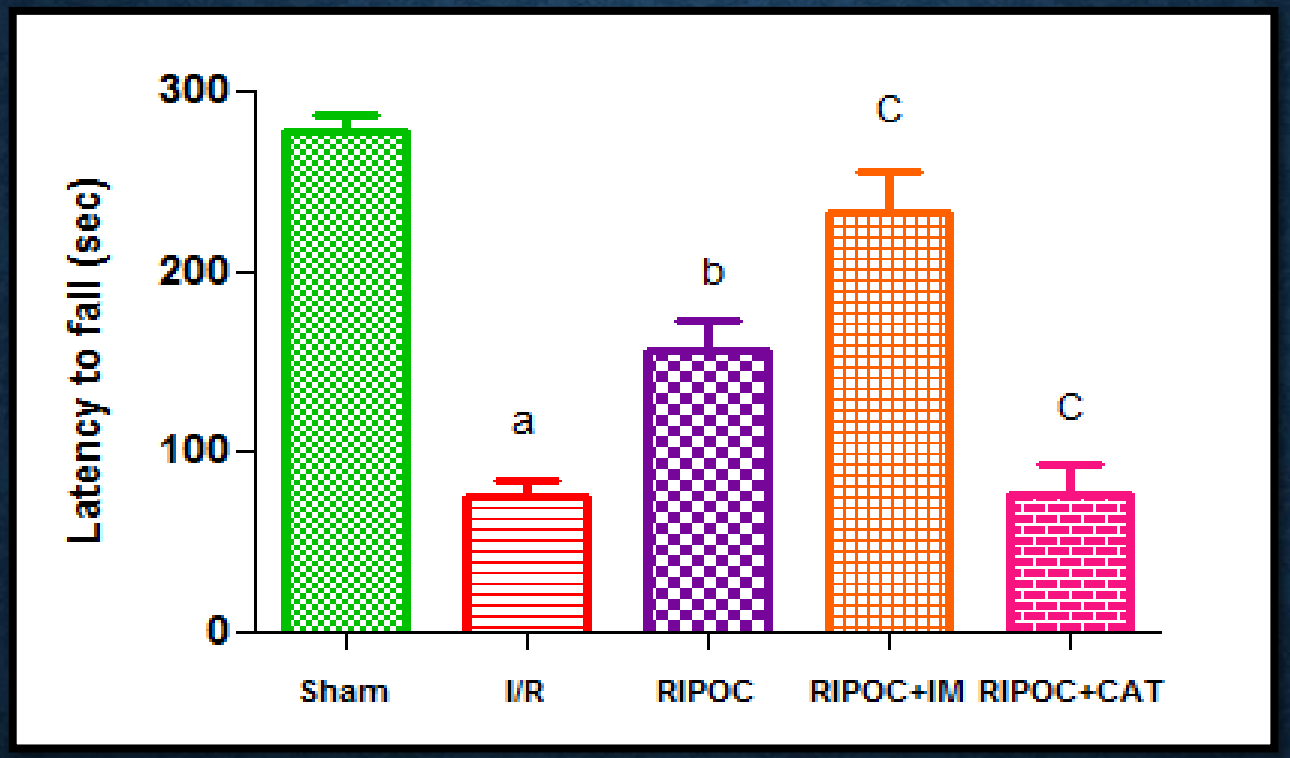


Fig.3. Effect of RIPOC, IM and CAT on rotarod performance.
[a]P<0.001 vs sham, [b]P<0.05 vs I/R, [c]P<0.05 vs RIPOC

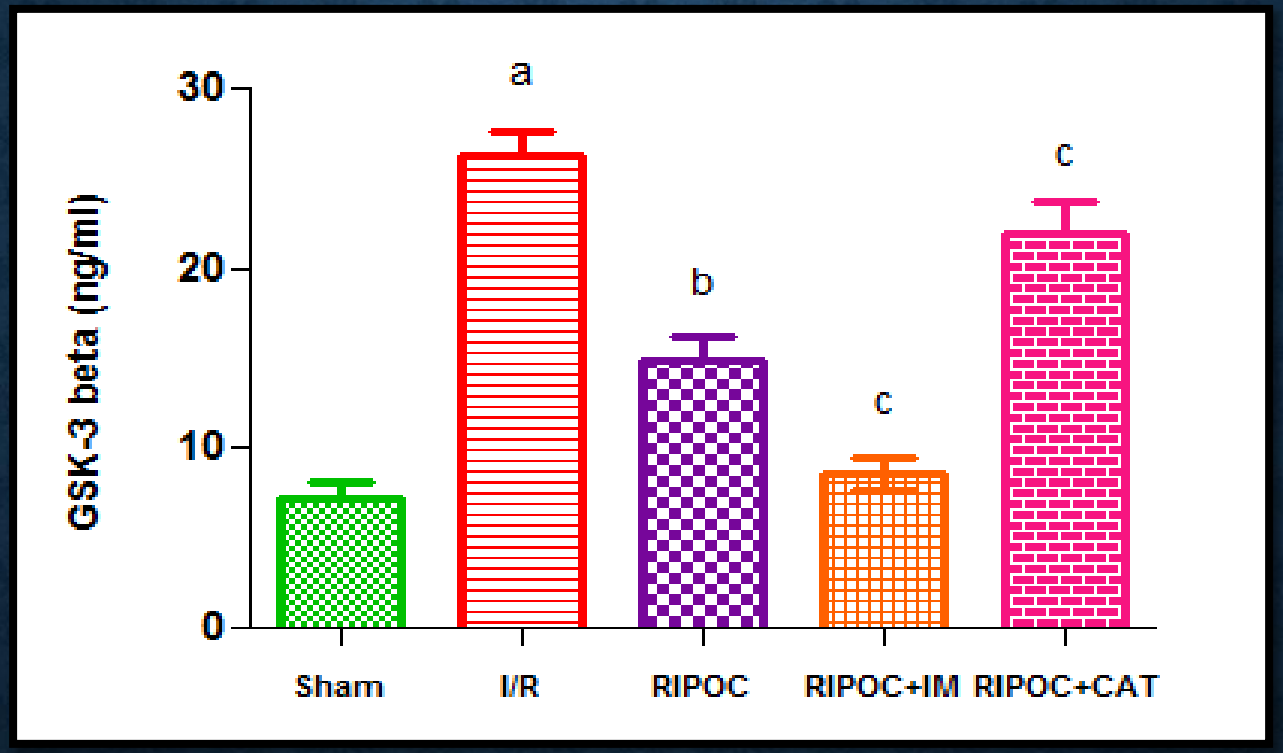


Fig.4. Effect of RIPOC, IM and CAT on GSK-3β activity.
[a]P<0.001 vs sham, [b]P<0.001 vs I/R, [c]P<0.05 vs RIPOC

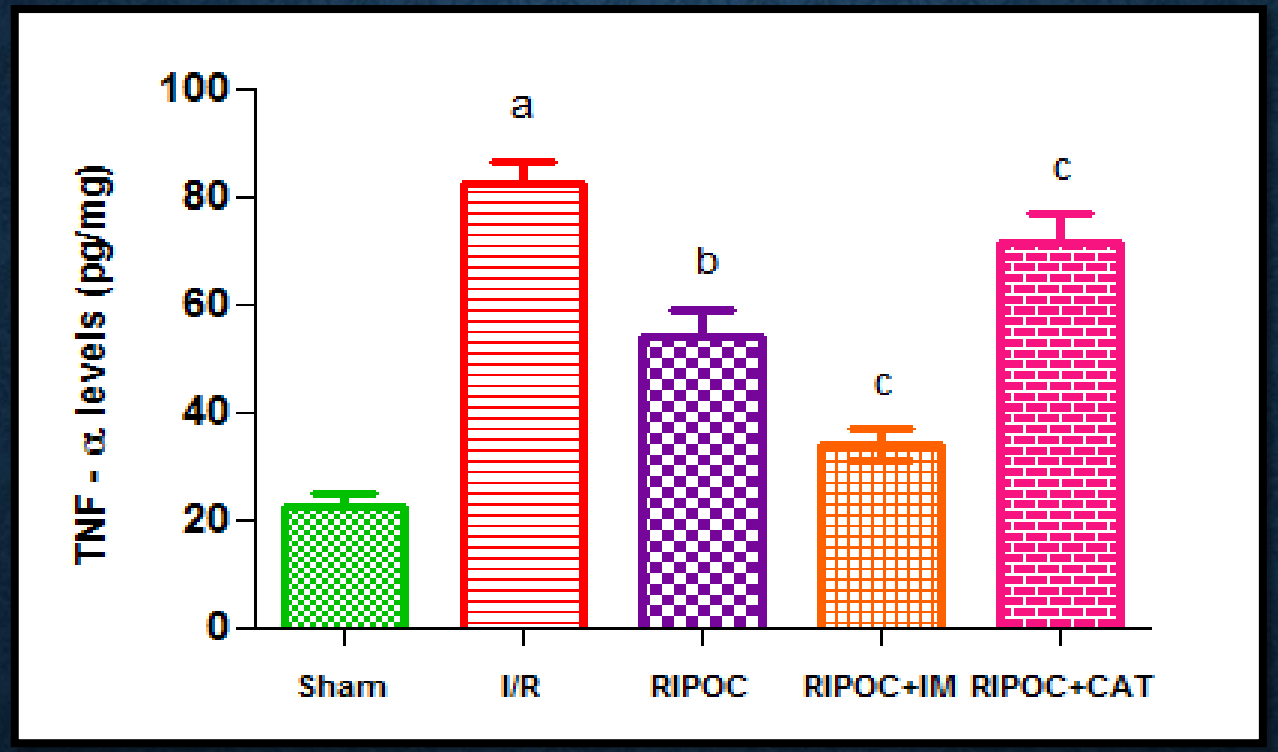


Fig.5. Effect of RIPOC, IM and CAT on TNF-α levels.
[a]P<0.001 vs sham, [b]P<0.001 vs I/R, [c]P<0.05 vs RIPOC

Groups	MDA (nMol/mg pro)	Nitrite (µMol/mg pro)	GSH (µMol/mg pro)	Catalase (U/mg pro)
Sham	77.25±21.34	7.15±0.94	1.52±0.06	1.24±0.15
I/R	267.1±29.04[a]	21.2±1.51[a]	0.37±0.08[a]	0.35±0.13[a]
RIPOC	172.2±17.03[b]	10.1±2.12[b]	1.14±0.16[b]	0.88±0.07[b]
RIPOC+IM	124.75±24.2	14.2±1.24	1.42±0.11[c]	1.17±0.12[c]
RIPOC+CAT	231.1±20.7[c]	5.64±0.84[c]	0.74±0.09[c]	0.67±0.1[c]

Fig.6. Effect of RIPOC, IM and CAT on biochemical parameters. [a]P<0.001 vs sham, [b]P<0.001 vs I/R, [c]P<0.05 vs RIPOC

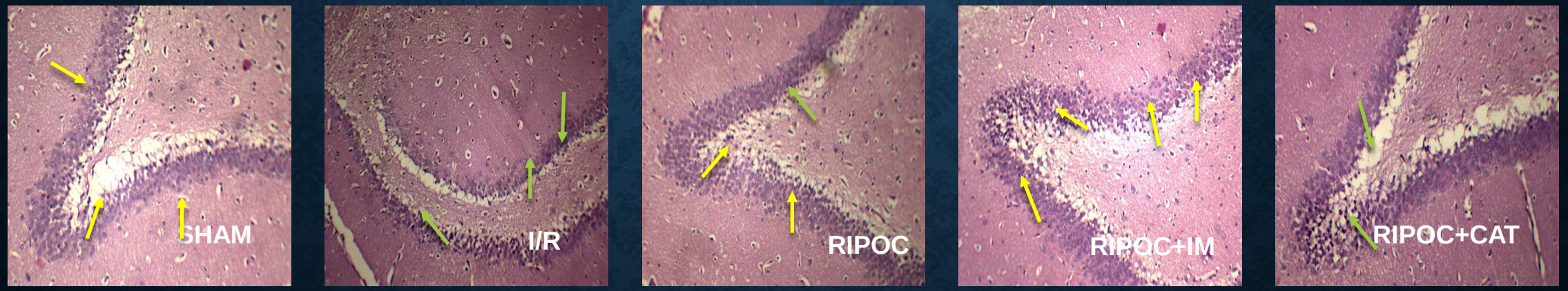


Fig.7. Effect of RIPOC, IM and CAT on dentate gyrus region of hippocampus

Discussion

- MCAO induced cerebral stroke resulted in severe neuronal injury as evidenced by behavioral and biochemical abnormalities

- RIPOC intervention significantly attenuated the motor impairment besides improving the biochemical and histological parameters

- Treatment with IM, before reperfusion provided synergistic neuroprotective role with RIPOC by inhibiting GSK-3β activity

- Opening of mPTP by CAT abolished the protective effect offered by RIPOC as evidenced by impaired motor performance, increased oxidative markers, neuroinflammation and high pyknotic count

Thus, the present study strongly indicates the therapeutic neuroprotective role offered by RIPOC against cerebral I/R injury is mediated by mPTP closing and GSK3β inhibition.

References

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