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Comparative Evaluation of Various Models of Ischemic Stroke in Rats

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Abstract: Stroke is defined as the loss of function of brain and it occurs when the supply of blood to the brain is either interrupted or reduced. When this happens, the brain does not get enough oxygen or nutrients which cause brain cells to die. Stroke induced brain injury results from the interaction of various complex pathophysiological modalities such as excessive accumulation of excitatory amino acids, ROS, calcium overload, mitochondrial damage, neuronal cell death etc. For the last three decades animal models of cerebral ischemia were developed with the aim of identifying mechanisms that cause tissue damage and to provide the basis for the development of new therapies for stroke at a preclinical level. The three main classes of *in-vivo* animal models are global ischemia, focal ischemia, and micro embolism/thrombosis model. *In vivo* experimental models to induce stroke are either global or focal models, each of them have their own advantages and disadvantages. In order to overcome the disadvantages, in the present study, we had combined both global and focal models i.e. Common carotid artery occlusion along with 25% $Fecl_3$ induced thrombosis. The model bilateral Common Carotid Artery Occlusion combined with Ferric chloride Induced Thrombosis and Reperfusion (BCCAO+FIT+RE) was more successful than the other groups such as Unilateral Common Carotid Artery Occlusion (UCCAO+RE), Bilateral Common Carotid Artery Occlusion (BCCAO+RE) and Ferric chloride Induced Thrombosis (FIT) in inducing stroke.
Keywords: Stroke, ischemia, thrombosis, occlusion, reperfusion

INTRODUCTION

The World Health organization (WHO) defined stroke as a focal neurological deficit (loss of function affecting a specific region of the nervous system) that occur due to disruption of blood supply to brain [1].This can be due to ischemia(lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a hemorrhage (leakage of blood). It is the third major cause of mortality and disability in the world.

Stroke induced brain injury results from the interaction of various complex pathophysiological modalities such as excessive accumulation of excitatory amino acids, ROS, calcium overload, mitochondrial damage, neuronal cell death etc. [2]. The molecular biology of stroke injury is a rapidly growingfield of research that may lead to the identificationof novel stroke targets and directed therapies. Mechanismsof CNS cell damage are determined experimentallyby testing effects of different manipulations on theextent of cell death in CNS brain cells*in vitro*, and in CNS tissue slice culture*in-vitro* models[3].

For the last three decades animal models of cerebral ischemiawere developed with the aim of identifying mechanisms that cause tissue damage and to provide the basis for the development of new therapies for stroke at a preclinical level [4]. Several recent animal models have been designed specifically to address specific risk factors, to determine neural repair processes, to test new treatment strategies. Today reliable animal models for stroke are available in a variety of species including primates, pigs, sheep, dogs, cats, Mongolian gerbils, rabbits, rats and mice.

The three main classes of *in-vivo* animal models are global ischemia, focal ischemia, and microembolism/thrombosis model. Technological advances and experimental discoveries have begun to define the cellular and molecular mechanisms involved in stroke injury. Explorations of these targets have led to the development of numerous agents that target various injury pathways [5].

However despite clear demonstration of numerous agents that can prevent the cascade of events leading to ischemicneuronal death in animal models, there is no obviousneuroprotective agent that has been shown to conclusivelyimprove stroke outcome in humans [6]. The inconsistencybetween animal results and clinical trials may bedue to several factors including: the heterogeneity ofhuman stroke, morphological and functional differencesbetween the

brain of humans and animals, the relativelylong post-stroke delay in administration of the drugs inclinical trials, and the better experimental control ofphysiological variables such as temperature, blood pressure and differences in evaluating efficacy in animal models [7].

There are a number of importantissues that remain unresolved regarding the translationof experimental developments to the clinical setting.The pharmacologist is therefore faced with a daunting number of models, none of which is known to be of predictive value[8]. Novel interventions will be required to overcome hurdlesassociated with bench-to-bedside translation and a new paradigm for drug developmentfor stroke treatment is required. Instead of focusing onsingle molecular targets on single cell types - which hasso far been a clinical failure, we suggest that targets withmore global signaling pathways and diverse cell loci reinvestigated [9].

As mentioned above, *in-vivo* experimental models to induce stroke either global or focal models, each of them have their own advantages and disadvantages. In order to overcome the disadvantages, in the present study, we are going to compare and evaluate different models of ischemic stroke.

MATERIALS AND METHODS

Experimental Animals

Albino rats of either sex weighing 150-250 g weight were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 25 ±20C 12 hr. light/dark cycle and given standard pellet diet and water. The animals were accustomed to the laboratory conditions for a week prior to the experimentation. Before using in experiment animals got clearance from IAEC, Anurag Pharmacy College, Kodad, Nalgonda, Telangana States (CPCSEA Registration No. 1712/PO/a/13/CPCSEA)

Experimental design

The animals were divided into five groups of six each as mentioned in table 1. Except the sham group all other groups' stroke is induced by different methods. The procedure for the induction of stroke has been described below.

Table-No. 1: Different models of ischemic stroke

Groups	Models
GROUP-I	Sham Group
GROUP-II	Unilateral common carotid artery occlusion (UCCAo)
GROUP-III	Bilateral common carotid artery occlusion (BCCAo)
GROUP-IV	Ferric chloride induced thrombosis (FIT)
GROUP-V	BCCAO+ FIT+ Reperfusion

Procedure for Induction of Stroke

Group 1: Sham group

The rats were subjected to surgical procedure but the arteries were neither occluded nor induced thrombosis. After 15 minutes the animals were sutured back and allowed to recover. After 72 hours, the rats were subjected to assessment of various behavioral and biochemical parameters.

Group 2: Unilateral common carotid artery occlusion (UCCAo)

The rats were anesthetized with i.m injection of ketamine (80-90 mg/kg i.m.) and xylazine (5-10 mg/kg i.m.). With the help of surgical kits the hair of upper central neck area was shaved and then an incision was made inorder to expose left common carotid artery, after the exposure the artery was doubly ligated for 15 minutes to produce partial global ischemia and cerebral reperfusion was allowed 72 h. Then animals were sutured and allowed to recover by housing them in individual cages. After 72 hours, the rats were subjected to assessment of various parameters as mentioned under methodology.

Group 3: Bilateral common carotid artery occlusion (BCCAo)

The rats were anesthetized with i.m injection of ketamine (80-90 mg/kg i.m.) and xylazine (5-10 mg/kg i.m.). With the help of surgical kits the hair of upper central neck area was shaved and then an incision was made in order to expose left and right common carotid arteries, after the exposure the arteries were doubly ligated for 15 minutes and cerebral reperfusion was allowed 72 h. Then animals were sutured and allowed to recover by housing them in individual cages. After 72 hours, the rats were subjected to assessment of various parameters

Group 4: Ferric chloride induced thrombosis (FIT)

The rats were anesthetized. Central neck area was shaved and then an incision was made inorder to expose left common carotid artery, after the exposure 25% $FeCl_3$solution was applied topically to the LCCA, and left for 15 min. Measures to be taken to avoid the contact of ferric chloride solution to other parts. Then animals were sutured and allowed to recover by housing them in individual cages.

Group 5: BCCAO+ FIT+ Reperfusion

The rats were anesthetized and incision was made on shaved central neck area in order to expose left common carotid artery which was double ligated for 15

minutes. After 15 min, 25% $Fecl_3$ was applied proximally to the surface of carotid artery. Ligation sutures were removed after 10 min of application of ferric chloride. In the same procedure is repeated for right CCA. Then animals were sutured and allowed to recover by housing them in individual cages.

Behavioral Parameters

All the animals were observed for their behavioral parameters after 72h of the surgery.

Torso Twisting

When rats are held by the tail above a flat surface a normal rat will extend the entire body toward the surface, rats with an infraction in show signs of body rotation. This rotation consists of mild twisting of the body to a severe body movement bringing the head and forelimbs into the vicinity of the hind limbs. Twisting was always toward the paralytic side [10].

Circling

Rats normally do not circle during normal state. Animals having infarction sometimes circle toward the contra lateral side [10].

Rota rod test

It was employed to evaluate forelimb and hind limb motor coordinationof animals [11].

Hole board test

This is the test to evaluate the exploratory behavior with help of an open field with holes on the bottom into which animals could poke their noses. Each animal was placed on the hole board and tested for 5 min before and after subjecting to the ischemia. The no. of counts for nose poking was calculated to evaluate the exploratory behavior [11].

Determination of intensity of ischemic damage by TTC staining

Randomly three animals in each group were selected and sacrificed 72 h after surgery by cervical decapitation. The brains were excised and stained with TTC inorder to identify the area of ischemia and intensity of damage. The brains were kept in freezer at 4˚C until they get hardened to cut into sections which were placed in 1% TTC (2, 3, 5 triphenyltetrazolium chloride) solution for half an hour. Then the sections stained with TTC were scanned for observing induction intensity of stroke [12-13].

Measurement of brain edema

Animals were sacrificed by cervical decapitation brain removed and weighed immediately to yield wet weight which is an indicator of brain edema.

Histopathological Examination

The brains from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5 µm thickness. The sections were stained with Heamotoxyllin and eosin dye for histopathological observation the change in inflammation structure was observed and compared.

***In vivo*brain antioxidant and prooxidant enzymes**

The remaining animals in each group were also sacrificed after 72 h post-surgery by cervical decapitation. The brains were excised and used for preparation of brain homogenate as follows:

Reagents

- 0.025 M sucrose solution: 85.87 gm. of sucrose was dissolved in 1000ml of distilled water.
- 10mM buffer solution: 1.2 gm. of tris was dissolved in 900 ml of distilled water, pH was adjusted to 7.4 with 1M HCl and dilute up to 1000ml.

Procedure

Brain was separated and kept in cold condition, were cross chopped with surgical scalpel into fine slices and was chilled in the cold 0.25 M sucrose, quickly blotted with filter paper. The tissue was minced and homogenized in ice cold 10 mM tris HCL buffer (to pH 7.4) at a concentration of 10% (w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so as to release soluble protein and leave only membrane and non-vascular matter in a sedimentable form. It was then centrifuged in cooling centrifuge at 5000 rpm for 20ºC, temperature was maintained at -4 ºC during the centrifugation, clear supernant was separated and used to estimate SOD, Catalase, Glutathione and Lipid peroxidation.

Super Oxide Dismutase (SOD)[14]

SOD was estimated by the method of Misra and Fridovich (1972)

Principle

Rate of auto oxidation of epinephrine and the sensitivity of this auto oxidation to inhibition by SOD were augmented as pH was raised from 7.8 – 10.2, O_2 generated by xanthine oxidase reaction, caused by the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O_2 introduced, the auto oxidation of epinephrine proceeds by at least two distinct pathways only one of which is free radical chain reaction involving O_2 and hence inhabitable by SOD.

Reagents

- Carbonate buffer (0.05 M, pH 10.2): 16.8 gm. of sodium bicarbonate and 22 gm. of sodium carbonate was dissolved in 500 ml of distilled water and the final volume was made up to with distilled water.

- Ethylene diaminetetra acetic acid (EDTA) (0.49 M): 1.82 gm. of EDTA was dissolved in 1000ml of distilled water.
- Epinephrine (3 mM): 9.9 mg of epinephrine bitartarate was dissolved in 10 ml of 1M HCL solution.
- SOD standard: Dissolve 1 mg (1000 units/mg) of SOD from bovine brain in 100 ml of carbonate buffer.

Procedure

0.5 ml of sample was diluted with 0.5 ml of distilled water, to this 0.25 ml ethanol, 0.5 ml of chloroform (all reagents chilled) was added, the mixture was shaken for one minute and centrifuged at 2000 rpm for 20 minute.

The enzymatic activity in supernatant was determined. To 0.05 ml of carbonate buffer (0.05 M, pH 10.2) and 0.5 ml of EDTA (0.49 M) was added. The reaction was initiated by the addition of 0.4 ml of epinephrine and the change in optical density/ min was measured at 480 nm. SOD activity was expressed as units / mg protein change in optical density/min. 50% inhibition of epinephrine to adrenochrome transition by enzyme is taken the enzyme unit. Calibration curve was prepared by using 10 – 125 units of SOD.

Calculation

$$SOD = \frac{(0.025 - Y)}{Y \times 50} \times 100$$

Where,

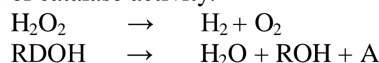
Y = Initial reading – Final reading

Catalase [15-16]

Catalase was estimated by Hugo E. Aebi method 1984.

Principle

In UV range H_2O_2 can be followed directly by the decrease in absorbance (O.D 240) per unit time is a measure of catalase activity.



$H_2O_2 \rightarrow H_2 + O_2$

$RDOH \rightarrow H_2O + ROH + A$

Decomposition of H_2O_2 = Decrease in absorbance at 240 nm.

Reagents

- Phosphate buffer: (50mM/1; pH 7.0)
  - ➢ Dissolve 6.81 gm. KH_2PO_4 in water and make up to 1000 ml.
  - ➢ Dissolve 8.9 gm. NaH_2PO_4 $2H_2O$ in water and make up to 1000 ml.
  - ➢ Mix the solutions (a) and (b) in proportion 1:15 (v/v)
- Hydrogen peroxide (30 mM/1): Dil 0.34 ml of 30 % hydrogen peroxide with phosphate buffer up to 100ml.

Procedure

Dilute homogenate 20 times with Phosphate buffer pH 7.0.

Table-2: Catalase procedure

Blank	Test
4 ml of homogenate diluted with 2 ml of phosphate buffer p^H 7, and take absorbance at 254 nm for 3 min. with 30 sec. interval	2 ml of homogenate diluted with 1 ml of H_2O_2 (8.5 micro lit. in 2.5 ml phosphate buffer (50mM/l. pH 7.0) and take the absorbance at 254 nm for 3 min. with 30 sec. interval.

Calculation

$$\text{Catalase} = \text{Log}\ (A / B) \times 2297.3$$

Where,

A: Initial absorbance

B: final absorbance (after 30 second)

Lipid Peroxidation[17]

Lipid peroxidation (Malondialdehyde formation) was estimated by Slater and Sawyer method (1971).

Reagents

- Thiobarbituric acid (0.67%) in 1M tris hydrochloride, pH 7, 0.67gm of thiobarbituric acid was dissolved in 100 ml of distilled water.
- Trichloroacetic acid (20%): 20 gm of trichloroacetic acid was dissolved in 100ml of distilled water.

Procedure

2 ml of sample was mixed with 2 ml of 20% Trichloroacetic acid and kept in ice for 15 min. The precipitate was separated by centrifugation and 2 ml of samples of clear supernatant solution were mixed with 2 ml of aqueous 0.67 thiobarbituric acid. This mixture was then heated with on a boiling water bath for 10 minute. It was cooled in ice for 5 minutes and absorbance was read at 535 nm. The values expressed as nm of MDA formed/mg of protein. Values are normalized to protein content of tissue.

Calculation

$$X = \frac{(Y + 0.002)}{0.0026086}$$

Where,

Y – Absorbance differences of final (after 3 min) and initial reading of test sample.

Statistical Analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dennett's test using computer based fitting program (Graph pad prism.5). Statistical significance was determined accordingly.

RESULTS

Behavioral Parameters

The behavioral parameters such as mobility, motor coordination, and exploratory behavior were assessed after 72 h of inducing stroke. The Results are given in table 3. The mobility of groups (II, III, IV& V) has been decreased when compared to sham operated group. In the group V animals, circling movement has been observed which indicates severe cerebral infarction due to combined effects of occlusion, thrombosis and reperfusion.

The motor coordination activity was measured with the help of rotarod apparatus. The group V showed significant (p<0.05 for water content and p<0.001 for motor coordination and exploratory behavior) decrease in motor coordination when compared to sham operated rats. The group II has shown a very slight reduction in motor activity when compared to group-I may be because of mild neurological deficit. The more the neurological deficit the less is the motor coordination.The results are shown in the table No. 3.

The exploratory behavior is assessed by hole board test. The ischemic insult decreases the exploratory behavior of the animal. The more the insult less is the no. of dips in the hole board by the rat. The average no. of dips of all stroke induced groups (II, III, IV and V) has been decreased significantly when compared with the sham operated group (I).

The cerebral water content or edema was increased significantly both in group III and Group V when compared to sham group.

Table-3: Representation of the effect of different models of stroke on brain water content, motor coordination and exploratory behavior of rats

Group No.	Stroke inducing model	Water content (%)	Motor coordination (sec)	Exploratory behavior (No. of dips)
I	Sham operated group (SOG)	62.35±2.70	109.50±4.33	36.15±1.75
II	UCCAO + Reperfusion (Re)	69.10±3.15^{ns}	85.78±4.28***	34.87±1.65^{ns}
III	BCCAO + Reperfusion (Re)	73.26±1.04*	47.51±2.33***	23.30±1.43***
IV	Ferric chloride induced thrombosis(FIT)	69.98±2.69^{ns}	59.25±1.91***	34.07±2.38^{ns}
V	BCCAO + FIT + Reperfusion (Re)	79.56±2.92**	26.34±1.09***	20.10±1.01***

All values are shown as mean ± SEM and n=6.
* indicate p<0.05, ** indicate p<0.01, *** indicate p<0.001, ns indicates non-significant when compared to controlgroup.

Brain antioxidant parameters

The biochemical results are showed in Fig- 1, 2, 3, 4 and Table- 5.The results showed that cerebral ischemia due to combined effects of thrombosis, occlusion and reperfusion in Group V significantly decreased antioxidant substances such as, SOD and CAT and increased the levels of lipid peroxidation in the injured brain tissue of rat when compared to sham operated group. In other inducing groups also there is incidence of increased levels of lipid peroxidation and decreased levels of antioxidant enzymes compared to sham group. But the prominent decreased values are seen in group V which indicates severe brain damage.

Table-4: Representation of the effect of different models of stroke on brain antioxidant parameters

Group No.	Stroke inducing model	SOD (Units/mg protein)	CAT (Units/mg protein)	LPO (nmoles/mg protein)
I	Sham operated group (SOG)	19.65±0.89	0.37±0.08	1.53±0.15
II	UCCAO +Reperfusion(Re)	17.32±0.94^{ns}	0.25±0.06^{ns}	2.30±0.51^{ns}
III	BCCAO +Reperfusion(Re)	12.47±0.85***	0.13±0.03*	6.89±0.74**
IV	Ferric chloride induced thrombosis(FIT)	16.42±0.94^{ns}	0.26±0.01^{ns}	4.13±0.99^{ns}
V	BCCAO+FIT+ Reperfusion(Re)	10.51±1.08***	0.07±0.01**	9.54±1.41***

All values are shown as mean ± SEM and n=6. * indicate p<0.05, ** indicate p<0.01, *** indicate p<0.001, ns indicates non-significant when compared to controlgroup.

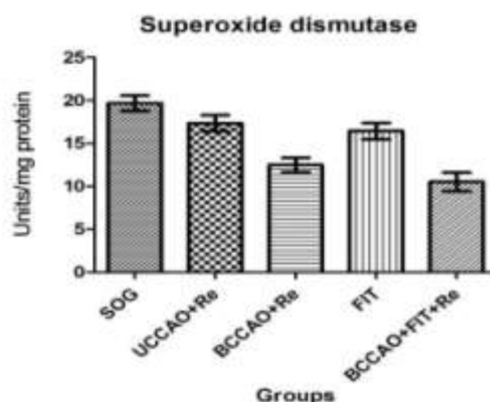


Fig-1: Representation of superoxide dismutase level

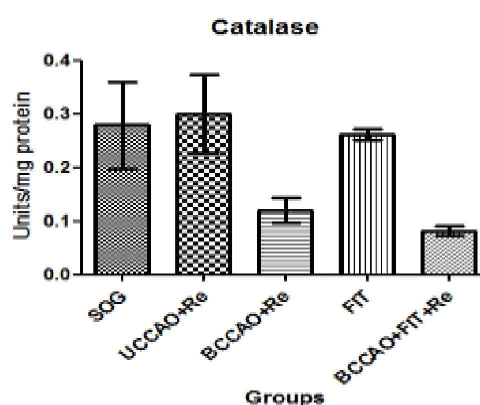


Fig-2: Representation of catalase level.

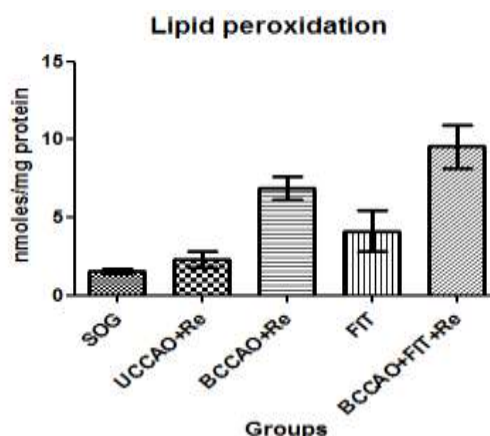


Fig-3: Representation of lipid peroxidation level

TTC Staining

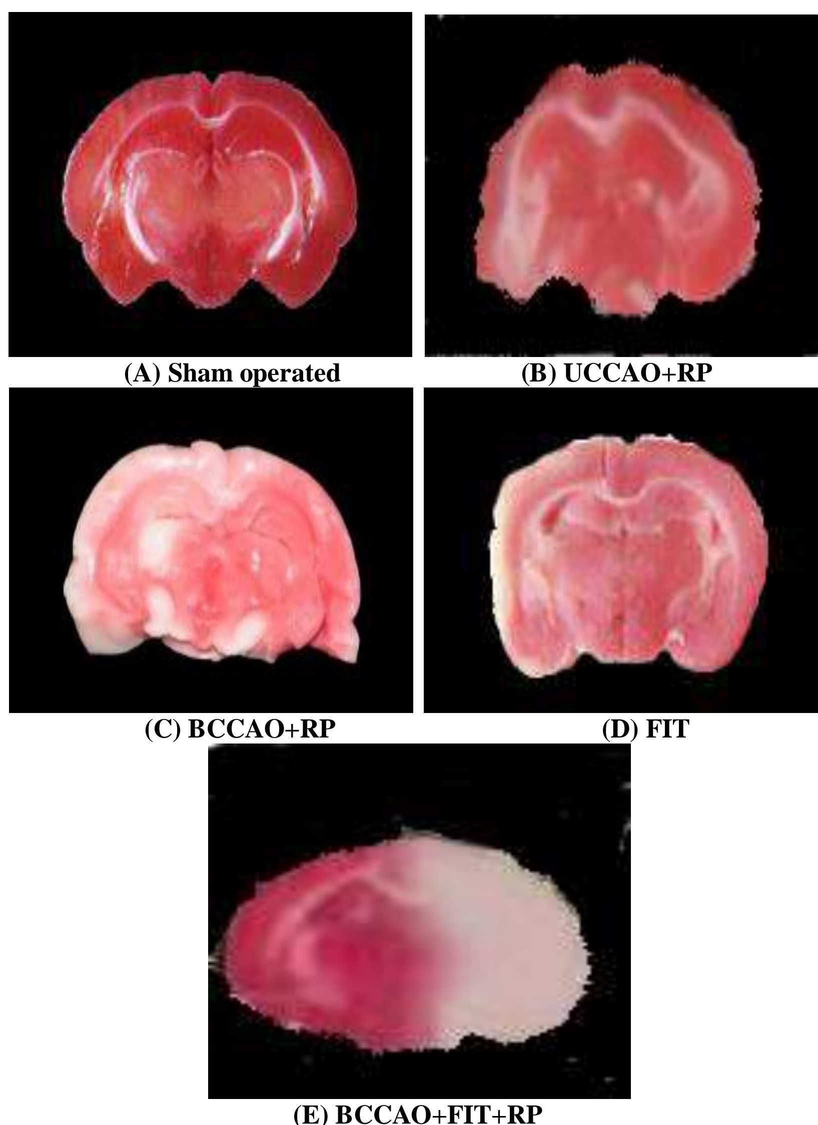
The infarction area in representative sections stained with TTC from different groups is shown in fig. 6.5, the large marked irreversible cerebral infarction area in hippocampus region is observed in Group V. The ischemic insult is less in group II AND Group IV. The damage is severe in group III.

Histopathology

The histopathology of the brain sections of various groups showed in Fig. 6.6. From the histopathological study, it was observed that Group V section (E) of brain tissue showing swollen neurons, dilated blood vessels with lymphocytic proliferation and neuronal necrosis occurred in brain regions induced by BCCAO+FIT+RP ischemic group shown in fig. 6.6 (E). While no apparent morphological changes and normal structure of brain section is seen in sham operated. In UCCAO (B) Group mild necrosis and foci of inflammation was observed. In (C) BCCAO section showed shrinkage, atrophy and necrosis of neurons along with vacuolization and inflammatory infiltration. There is significant necrosis observed in (D).

Observation

The sham operated group (A) maintained the normal architecture. Mild necrosis and foci of inflammation was observed in (B). Ischemia (C) caused marked congestion of blood vessels and neutrophil infiltration and increased intracellular spaces. These effects were further augmented by reperfusion and ferric chloride application i.e. lymphocytic proliferation and neuronal necrosis in E. There is significant necrosis observed in (D).

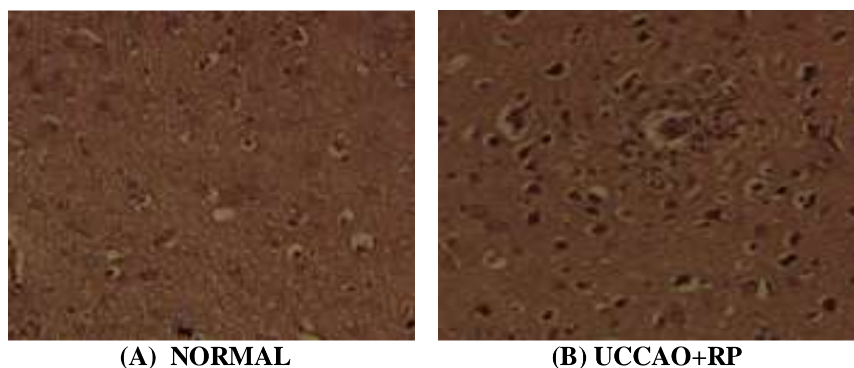


(A) Sham operated **(B) UCCAO+RP**

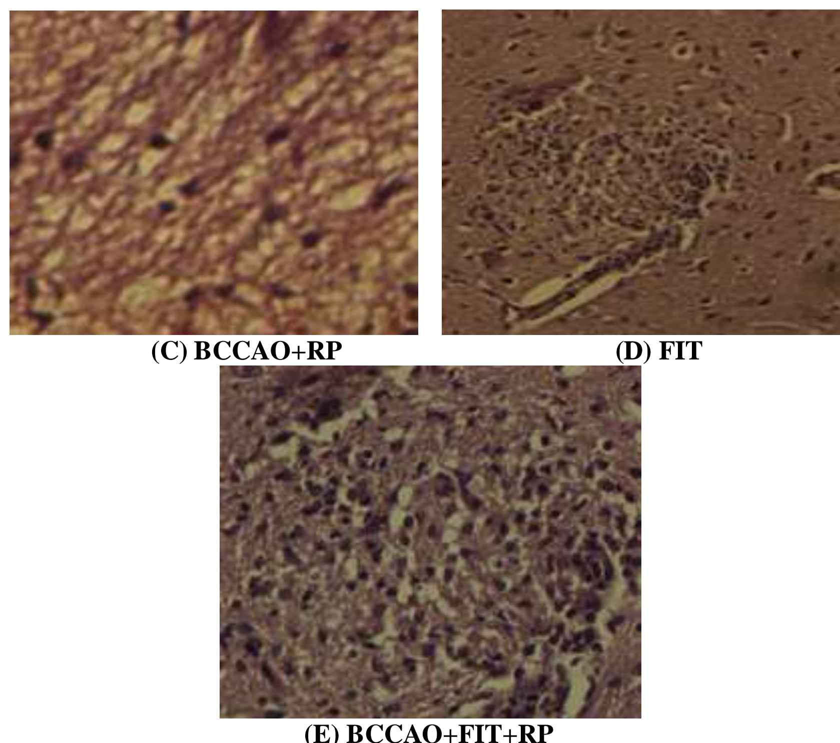
(C) BCCAO+RP **(D) FIT**

(E) BCCAO+FIT+RP

Fig-4: The effect of different induction models against global cerebral ischemia/reperfusion damage in rats evaluated by 2, 3, 5-triphenyltetrazolium chloride (TTC) staining. Brain coronal sections were prepared (2mmthickness) and then each section was stained with TTC



(A) NORMAL **(B) UCCAO+RP**



(E) BCCAO+FIT+RP

Fig-5: The effect of different induction models against global cerebral ischemia/reperfusion damage in rats. Photographs of brain sections from different groups stained with Heamotoxyllin & Eosin (A): Sham operated; (B): UCCAO+RP; (C) BCCAO+RP; (D) FIT; (E) BCCAO+FIT+RP

DISCUSSION

In vivo experimental models to induce stroke are either global or focal models, each of them have their own advantages and disadvantages. In order to overcome the disadvantages, in the present study, we had combined both global and focal models i.e. Common carotid artery occlusion along with 25% $Fecl_3$ induced thrombosis [18].

The exploratory behavior was very significant in Group V animals and in other groups it was not that much significant. Motor coordination activity was also measured and it was found that the group V animals have decreased motor activities when compared to sham operated group.

Lipid peroxidation can inhibit the function of membrane bound receptors and enzymes. The thiobarbituric acid reacting substance (TBARS) assay is used as an indicator of lipid peroxidation and levels of free radicals. The assay is based on the reactions of thiobarbituric acid with malondialdehyde produced during lipid peroxidation. As observed in our study, the stroke induced groups showed increase in malondialdehyde in brain affected by ischemic-reperfusion injury which suggested enhanced lipid peroxidation. The combined effect of occlusion thrombosis and reperfusion drastically increased the lipid peroxidation because of its combined effects [19].

Superoxide dismutase is an important endogenous antioxidant and prevents production of free radicals. The group V showed a decreased level of SOD as compared to sham operated group. Catalase decomposes hydrogen peroxidase and converts it to water and diatomic oxygen, whereas superoxide dismutase generates H_2O_2 from free radicals. An increase in production of superoxide dismutase without a subsequent elevation of catalase leads to the accumulation of hydrogen peroxidase, which is converted to hydroxyl radicals that produced deleterious effect on brain. In the present study, catalase levels were found to be less in animals subjected to ischemic-reperfusion injury. The reactive-oxygen-species-induced lipid peroxidation causes more production of hydroxyl radicals, which then inactivates catalase [20].

Reduced Glutathione (GSH) is one of the primary endogeneous antioxidant defense systems in brain, which removes hydrogen peroxides and lipid peroxidase. Decline in GSH levels could either increase or reflect oxidative state. In present study decreased GSH levels were observed hippocampus of ischemic rats. Depletion of GSH level in BCCAO+FIT+RP group as compared to sham operated group signified the same thing. It has been shown that depletion in GSH levels in ischemic reperfusion injury can be attributed to several factors such as cleavage GSH levels to cysteine, decrease in synthesis of GSH and formation of mixed disulfides, causing their cellular stores to be depleted [21].

2, 3, 5-triphenyltetrazolium chloride (TTC) is converted to red formazone pigment by nicotinamide

adenine dinucleotide (NAD) and dehydrogenase present in living cells. Hence viable cells were stained deep red. The infarcted cells loose these enzymes and, thus, remained unstained dull yellow. The BCCAO control group thus showed higher cerebral infarction due to increased cell death, which was found to be reduced inother groups.

Ischemic-reperfusion injury is known to produce necrosis of brain, which can be directly visualized by histological study; biopsy of the rat brain subjected to ischemic-reperfusion injury showed significant necrosis. But the group V has significant necrosis, neutrophil infiltration and increased intracellular spaces. The severe neuronal loss, observed as shrinkage of neurons and atrophy, was observed in Histopathological sections of ischemic re perfused brains.

CONCLUSION

From the above conducted study it can be concluded that the group V i.e. Bilateral Common Carotid Artery Occlusion combined with Ferric chloride Induced Thrombosis and Reperfusion (BCCAO+FIT+RE) was more successful than the other groups such as Unilateral Common Carotid Artery Occlusion (UCCAO+RE), Bilateral Common Carotid Artery Occlusion (BCCAO+RE) and Ferric chloride Induced Thrombosis (FIT)in inducing stroke. A significant change has been found in various evaluation parameters in the successful groupwhich may be beneficial for the better understanding of pathophysiology of stroke in order to develop an effective drug for the better treatment of ischemic stroke. Further study has to be carried out for the well development of stroke models from which the exact pathophysiology can be understood and processed for the new drug development for the treatment of stroke.

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