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EVALUATION OF MEDICINAL DRUGS ON IN- VIVO ANTI OXIDANT AND NEUROBEHAVIORAL IN RATS

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ABSTRACT

To study the comparative and combinational effects of Aspirin and N-Acetylcysteine on global cerebral ischemic reperfusion injury. The combination of ASP and NAC improved neurological outcome compared with NAC and ASP given alone but the difference is not significant so there is no significant additive effect was observed. The improvement in anti-oxidant potential of combinational treatment group shown significant difference with individual treatment groups.

Key Words: comparative and combinational effects, neurobehavioral

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INTRODUCTION

Ischemic stroke is a major cause of death and disability all over the world. It is the third leading cause of death, after heart disease and cancer, and the leading cause of long-term disability in major industrialized countries. Ischemic stroke results from a temporary or permanent reduction of cerebral blood flow that leads to functional and structural damage in different brain regions. Cellular damage occurs during ischemia and reperfusion (Reflow of blood with high pressure) [1]. Among all ischemic strokes, global cerebral ischemia is a major problem of experimental and medicine and biology. Cerebral ischemic-reperfusion is induced mainly by neuronal injury and death is not completely known. The brain is Particularly vulnerable to ischemia. Complete interruption of blood flow to the brain for only 5 minutes triggers the death of vulnerable neurons in several brain regions, whereas 20 minutes of ischemia is required to kill cardiac myocytes or kidney cells [2].Global Cerebral ischemia-reperfusion injury (GCIRI) is well-known that hippocampus, especially CA1 area, is vulnerable during global cerebral ischemia, therefore, learning and memory impairments may occur in many of these patients due to hippocampal damage and despite many efforts, there is no effective approach to prevent and treat this memory and cognitive impairment. Many studies have demonstrated that, inflammation following cerebral ischemia and reperfusion after brain ischemia, increases these inflammatory reactions, which can exacerbate neuronal injury. Release of pro-inflammatory cytokines, such as Tumour necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6, during ischemia/ reperfusion injury following both focal and global cerebral ischemia and production of these cytokines can significantly increase the risk and extent of brain injury. A cascade of cellular and molecular

events during brain ischemia, leads to delayed neuronal death[4]. The present study was intended to evaluate the possible neroprotective effects of N-acetyl cysteine (NAC) and Aspirin (ASP) against global cerebral ischemia by evaluating biochemical , behavioural and histopathological studies.5-Lipoxygenase (5-LO), a key enzyme metabolizing arachidonic acid to produce leukotrienes, has been reported to be involved in brain injury[1]. Growing evidence supports the role of oxidative stress as one of the primary factors in brain injury mediated by cerebral ischemia and stroke. The brain is particularly vulnerable to oxidative stress because of its high rate of oxidative metabolic activity, intense production of reactive oxygen species (ROS) metabolites. ROS namely superoxide and hydroxyl free radicals, together with hydrogen peroxide have been proposed to cause neurotoxic effect and initiate a free radical-mediated chain reaction causing additional damage to diverse areas in the brain. Therefore, oxidative injury could be one possible cellular cascade affecting all organs and tissues during ischemia[3, 4].Many antioxidants are reported to reduce ROS-mediated reactions and rescue neurons from ischemia/reperfusion induced neuronal loss in animal models of cerebral ischemia. Over depolarization may also cause neuronal death this is due to the energy loss, pathophysiology is as follows: energy failure results in neuron depolarization, which causes activation of glutamate receptors, which in turn alters ionic gradients of Na+, Ca++, Cl-, and K+. As glutamate increases in the extracellular space, peri-infarct depolarization occurs. Then, as water shifts occur, cells swell with resulting cerebral edema. The result of increasing intracellular Ca++ is an upregulation of a variety of enzyme systems such as lipases, proteases, and endonucleases. As a result, free O_2 radicals are generated via a variety of biochemical pathways, and apoptotic cell death occurs[5, 6].

MATERIALS AND METHODS

Animals

Rats weighing 250-300 grams were obtained from Mahaveer Enterprises, Hyderabad, Andhra Pradesh, India. Animals were maintained under standard conditions 12 h light/dark cycle; 25 ± 3^0C, 45–65% humidity and given standard rat pellet feed and water ad libitum. Animal studies were performed according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India.

Preperation of Homogenate

The brains were removed after observing behavioural tests. The brain was homogenized in cold phosphate-buffered saline (10% w/v) and made suspension by using tissue homogeniser. The suspension was centrifuged at 7000 rpm for 25 min at -4^{0}C, supernatant is formed. The supernatant was used for the following biochemical analysis [7].

Estimation of Lipid Peroxidation

The extent of lipid peroxidation was measured by estimating the amount of malanodialdehyde (MDA) formed. The principle of this method is based on the spectrophotometric measurement of the color that occurs during the reaction of thiobarbituricacid with MDA. The concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of the malondialdehyde thiobarbituric acid complex and expressed in nmol MDA/mg protein. Procedure involved for measurement of lipid peroxidation is 0.1 ml homogenate is taken, to that 2 ml of mixture of 0.25N HCL, 15% (w/v) trichloroacetic acid (TCA) and 0.37% (w/v) thiobarbituric acid were added and the mixture in boiling water bath for 30 min and it is placed in crused ice for 10 min and centrifuged at 7000 rpm for 10 min. Pink colour supernatant is formed and observance measured at 532 nm and the results were expressed as n mol MDA/mg protein [8].

Estimation of Reduced Glutathione

To 0.5ml of homogenate add 0.5ml of 5% of Trichloroacetic acid. Centrifuge at 3000rpm for 20 min at 4^{o}C. Take 0.1 ml of supernatant from the above mixture, To this 1ml of Sodium phosphate buffer of 0.2M P^H 8 and 0.5 ml of 0.6mM DTNB reagent in 0.2M Phosphate buffer saline were added. Then formation of yellow colour and absorbance was measured at 412 nm. Results were expressed as nmol/mg protein [9].

Statistical Analysis

The values were expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Tukey's test using Graph pad prism software. Statistical significance was set at P≤0.05.

RESULTS AND DISCUSSION

Sham control group of animals weren't shown significance difference in comparison with control group of animals.MDA level in the BCCA occluded group of animals shown significant (P<0.001) increase in comparison with sham control group of animals. Treatment groups NAC, ASP and combinational treatment groups shown significant (P<0.001) decrease in MDA levels when compared with BCCA occluded group of animals. The individual treatment groups ASP alone shown significant difference with combination treatment group. All the data was tabulated in table no (1).

Table No-1 Estimation of MDA Levels

S.NO	Groups	MDA(mM / g wet tissue) Mean ± SEM
1	Control	1.57±0.027
2	Sham control	1.76±0.026
3	BCCA ligation control	3.78±0.061***
4	Treatment with NAC	1.72±0.0551***
5	Treatment with ASP	1.94±0.086***
6	Treatment with NAC and ASP	1.53±0.041***

Sham control group of animals weren't shown significance difference in comparison with control group of animals. Glutathione levels in the BCCA occluded group of animals shown significant (P<0.001) decrease in comparison with sham control group of animals. Treatment groups NAC, ASP and combinational treatment groups shown significant (P<0.01), (P<0.001) and (P<0.001) increase in glutathione levels respectively when compared with BCCA occluded group of animals.The combination treatment group showed significant difference (P<0.05) with NAC alone treatment group but not with ASP alone treatment group. All the data was tabulated in table no (2)

Table No-2 Estimation of Reduced Glutathione

S.NO	Groups	Glutathione (mM / g wet tissue) Mean ± SEM
1	Control	4.90±0.153
2	Sham control	4.43±0.273
3	BCCA ligation control	2.47±0.145***
4	Treatment with NAC	3.57±0.120**
5	Treatment with Aspirin	3.87±0.088***
6	Treatment with NAC and ASP	4.40±0.057***,#

CONCLUSION

This study suggesting that the combinational treatment group was able to improve behavioual performances and also anti-oxidant potential.The additive effect wasn't observed in behavioural performances whereas additive effect was observed in combinational group for the potentiation of anti-oxidant defence system than individual treatment groups.

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