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EVALUATION OF THE COMBINATION OF TWO HERBAL DRUGS IN MIDDLE CEREBRAL ARTERY OCCLUSION INDUCED BRAIN STROKE IN RATS

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ABSTRACT

Stroke is defined as brain injury which is caused by a sudden obstruction in the blood circulation to the brain. When blood supply altered/arrested about two million brain cells die in each second, which can increase the risk of brain damage, disability and death. Present study focused on the fact that the combination of the hydro alcoholic extract of *Eclipta alba* and *Centella asiatica* can reverse MCAO induced brain stroke, protect brain form stroke and protect brain from other complication which are precipitated by brain stroke. In MCAO treated group MDA level was increased significantly and GSH was significantly decreased compare to control and SHAM group. These altered biochemical parameters were restored to normal values significantly respectively by administering hydro alcoholic extract of combination of *Eclipta alba* and *Centella asiatica* extract and melatonin. The results of present study suggest that the combination of *eclipta alba* and *centella asiatica* is more potent than individual drug treatment, and contains oxidative stress reducing properties, neuroprotective property and prevent brain stroke as well as minimize the symptoms of stroke.

KEYWORDS: Stroke, brain, oxidative stress, neuroprotective, ischemia.

1. INTRODUCTION

The third major cause of death is stroke[1] after cardiovascular disease and cancer in the major industrialized countries.[2,3] The World Health Organization **(**1970s**)** explained stroke as a

"neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours", although the word "stroke" is centuries old. It was founded that stroke was responsible for 5.5 million deaths and 15 million nonlethal brain injuries worldwide; in 2001, these figures are projected to increase to 6.3 million deaths in 2015 and 7.8 million in 2030.[4] Stroke lethality is 11% in women and 8.4% in men and it was also seen that this is more prevalent among blacks than whites, especially in the younger age groups.[5] Among which, 88% are ischemic, 9% involve an intracerebral hemorrhage and remaining 3% involve a subarachnoid hemorrhage. As per United States report, approximately 700,000 strokes occur each year[6] most of which are caused by a blockage in blood flow.[7] High polyunsaturated fatty acid content in brain is susceptible to ROS damage.[8] Superoxide and hydroxyl radicals are deemed to cause major devastation to the cell membrane by lipid peroxidation among all of the produced free radicals.[9]

2. MATERIAL AND METHODS

2.1. Animals and housing conditions

All animal experiment protocols were approved and conducted according to the guideline of Institutional Animal Ethics Committee (IAEC) protocol numbered (1149/PO/ac/07/ CPCSEA). Male wistar rats (animal facilitation center RV Northland Institute, Dadri, Greater Noida) weighing between 250-350g.and the rats were 2-3 months old. Animals were housed at 22 ± 2°C and allowed free access to food and water. 12 hr. dark and 12 hr. light conditions was maintained.

2.2. Chemicals Used

2,3,5- Triphenyltetrazolium (TTC), Acetic Acid (AA), Diethyl ether, Ethanol (100%), Formaldehyde, Glacial acetic acid, Paraffin wax, Sodium bicarbonate ($NaHCO_3$), Sodium Dodecyl Sulphate (SDS), Sodium dihydrogen orthophosphate (NaH_2PO_4), Disodium phosphate (Na_2HPO_4), Di-Ethylene tetra acetic acid (EDTA), Sodium chloride (NaCl) were purchased from CDH. Nylon monofilament (3-0&4-0) was supplied by Ethicon, Johnson and Johnson. Bovine serum albumin (BSA), poly-L-lysine (0.1% w/v), Folin reagent, Sulphosalisylic acid hydrate, Thiobarbaturic acid (TBA), 5,5'-Dithiobis (2-nitrobenzoic acid)/ Ellman's reagent, were all purchased from sigma Aldrich. Ketamine hydrochloride, Diazepam (Aniket) was purchased from Neon Laboratories.

2.3. Preparation of extract

Dried whole plant of Bhringraj *(eclipta alba*) (Ref No-NISCAIR/RHMD/Consult/2015/2808/1-2) and Brahmi *(centella asiatica)* (Ref No-NISCAIR/RHMD/Consult/2015/2808/1-1) were procured from market khari baoli, new dellhi, and was authenticated by Dr. Sunita Garg, chief scientist & head raw materials & museum, (rhmd), niscair, new delhi. Dry leaf powder of *eclipta alba* was suspended in 50% ethanol in ratio 1:3 and was stirred overnight at 50°C, followed by filtration under sterile conditions. To remove the solvent completely filtrate was vaccum dried at 50°C, weighed and reconstituted in water to 50 mg/ml EAE. The extract yield was 11.6% (w/w) and it was stored at -20°C in 1 ml aliquots until further use.[10] The dried powder of *Centella asiatica* was extracted with 95% methanol. To obtain the extract, the soxhletion was done for a week. After that, the Extract was evaporated in water bath at 50°C to obtained crude for antioxidant assay.[11]

2.4 Grouping of animals

The animals were divided into four major groups, such as,

Group I- Control (Normal saline, 1ml PO for 15 days)

Group II- Normal saline (1ml PO for 15 days) + SHAM treatment

Group III- MCAO*+ (Normal saline, 1ml PO)

Group IV- Standard (melatonin 30mg/kg PO) + MCAO*

Group V- 1ml of *Eclipta alba* extract PO (500mg/kg) + MCAO*

Group VI-1ml of *Centella asiatica* extract PO (500mg/kg) + MCAO*

Group VII- Combination of *Eclipta alba* and *Centella asiatica* (1ml extract of EA (250mg/kg) PO+ 1ml extract of CA (250mg/kg) PO) + MCAO*

Where ***** - after 24 hr. of 15 day dosing MCAO procedure perform.

2.5 Experimental Design

2.5.1. Method Induction-After turning the animal to the supine position, it was fixed to the surgical table using an adhesive tape. A midline neck incision was made and the soft tissues over the trachea were retracted gently. The common carotid artery (CCA) either left or right was carefully isolated from the vagous nerve and ligated temporarily using a cotton thread. Generally, the CCA was bifurcated in to the external carotid artery (ECA) and internal carotid artery (ICA) which flows toward the head region, and again bifurcated into the MCA and pterygopalatine artery. Then, two closely spaced permanent knots were placed at the distal part of the ECA to prevent the backflow of blood and the ECA was cut between the

knots. The tied section attached proximal to the CCA junction could best straightened to allow the filament to enter the ICA, and then, the second bifurcation was cleared to obtain a good view of the MCA. The micro vascular clip was placed in the ICA temporarily proximal to the CCA junction and the tied section of ECA was incised using the micro scissors to insert the monofilament.

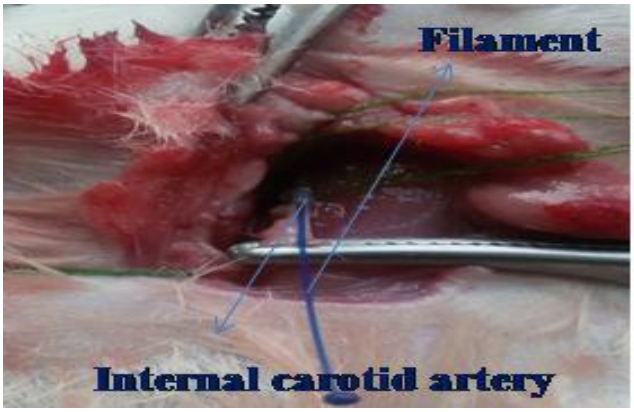

Figure 1: Middle cerebral artery occlusion induced method.

Once the tip of the monofilament reaches the Common carotid artery (CCA) junction, a knot was placed below the arteriotomy in the ECA, and then, the micro vascular clip which was placed in the internal carotid artery (ICA) was removed permanently to allow filament insertion. The ECA stump was straightened and the filament was advanced carefully up to 17–20 mm, for rats, in to the MCA from the CCA junction. After the specific occlusion period, again a clip was placed in the ICA and the knot placed in the external carotid artery (ECA) stump below the arteriotomy was loosened. The intensity of the infarction greatly depends on the MCA (middle cerebral artery) occlusion period. The minimum 2hr. of the occlusion period was required to obtain are producible infarct volume, the filament was then withdrawn carefully until the tip was near the arteriotomy.

After the removal of the filament, the knot was tightened in the ECA. After the confirmation the midline neck incision was sewed using surgical suture.

At the end point of the study, the animals were sacrificed and the histological analysis was carried out to confirm infarction. Generally, brain infarction could be observed after 24 h of reperfusion or surgery.[12]

2.5.2. Dose Selection-The ketamine (80 mg/kg) and Diazepam (2.5 mg/kg) intra-peritonealy or induced and maintained with 3 % and 1.5 % isoflurane, respectively, were used to anesthetize the animals.

3. RESULT

Effect of *Eclipta alba* (*Eclipta alba*) extract *Centella asiatica* (*Centella asiatica)* extract and the combination of (*Eclipta alba and Centella asiatica*) extract on infarct area in middle cerebral artery occlusion induced brain stroke in experimental animal rats.

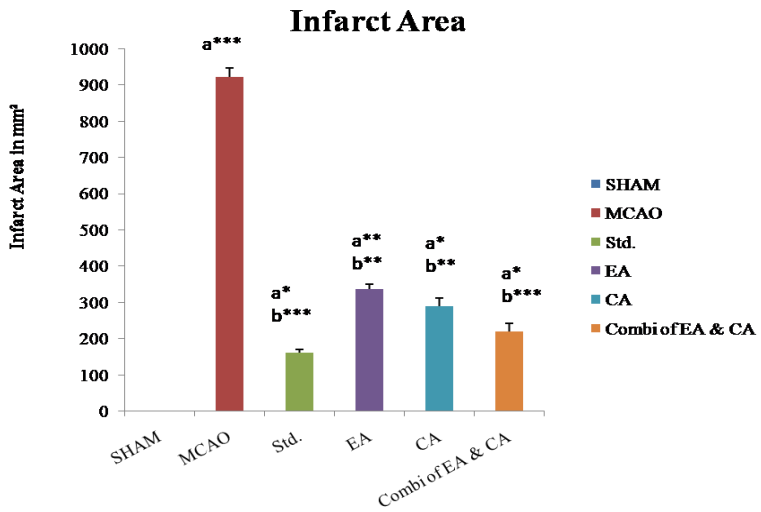

Figure 2: Effect of combination of *Eclipta alba* and *Centella asiatica* on infract area in middle cerebral artery occlusion induced brain stroke in experimental animal rats. All values are expressed as Mean ± SEM. ***p = 0.001, **p<0.01, *p<0.05. a *vs.* SHAM, b *vs.*MCAO.

Effect of *Eclipta alba extract*, *Centella asiatica extract* and the combination of (*Eclipta alba and Centella asiatica*) extract on neurological evalution in middle cerebral artery occlusion induced brain stroke in experimental animal rats.

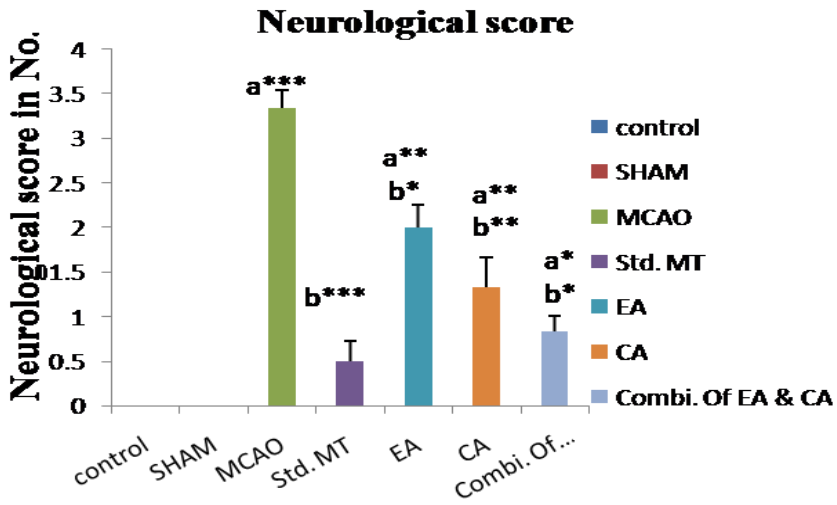

Figure 3: Effect of combination of *Eclipta alba* and *Centella asiatica* on neurological score in middle cerebral artery occlusion induced brain stroke in experimental animal rats. All values are expressed as Mean ± SEM. ***p = 0.001, **p<0.01. a*vs.* SHAM,b *vs.*MCAO.

Effect of *Eclipta alba* (*Eclipta alba*) extract, *Centella asiatica (Centella asiatica)* and the combination of (*Eclipta alba and Centella asiatica*) on glutathione (GSH) level in middle cerebral artery occlusion induced brain stroke in experimental animal rats.

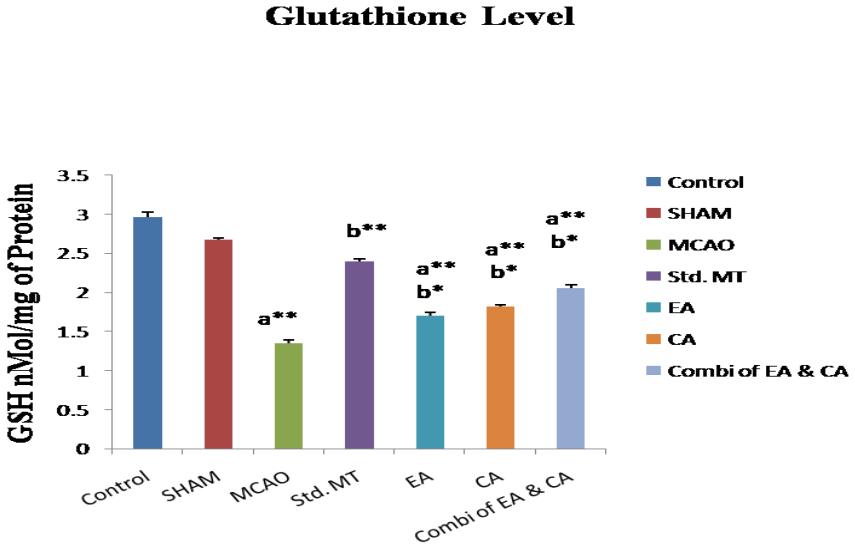

Figure 4: Effect of combination of *Eclipta alba* and *Centella asiatica* on GSH level in middle cerebral artery occlusion induced brain stroke in experimental animal rats. All values are expressed as Mean ± SEM. ***p = 0.001, **p<0.01. a*vs.* SHAM, b *vs.*MCAO.

Effect of *Eclipta alba* (*Eclipta alba*) extract, *Centella asiatica (Centella asiatica)* extract and combination of *Eclipta alba* and *Centella asiatica* extract on lipid peroxidation (MDA) level in middle cerebral artery occlusion induced brain stroke in experimental animal rats.

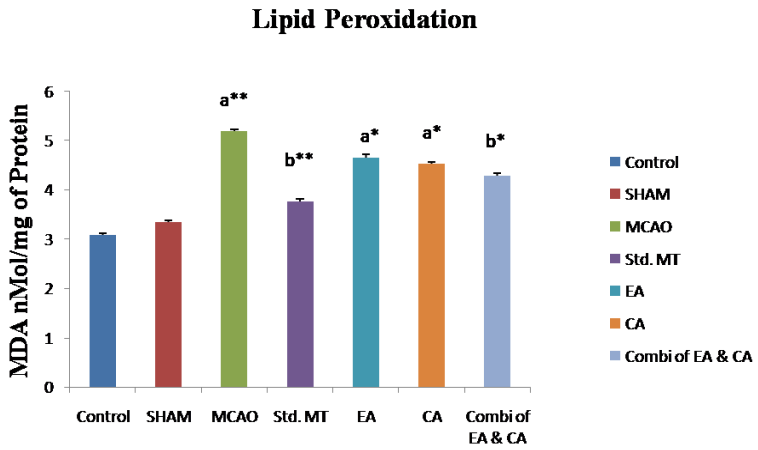

Figure 5: Effect of combination of *Eclipta alba* and *Centella asiatica* on MDA level in middle cerebral artery occlusion induced brain stroke in experimental animal rats. All values are expressed as Mean ± SEM. ***p = 0.001, **p<0.01. a*vs.* SHAM, b *vs.*MCAO.

Effect of *Eclipta alba (Eclipta alba)* extract, *Centella asiatica (Centella asiatica)* and the combination of (*Eclipta alba and Centella asiatica*) on sensory motor function in middle cerebral artery occlusion induced brain stroke in experimental rats.

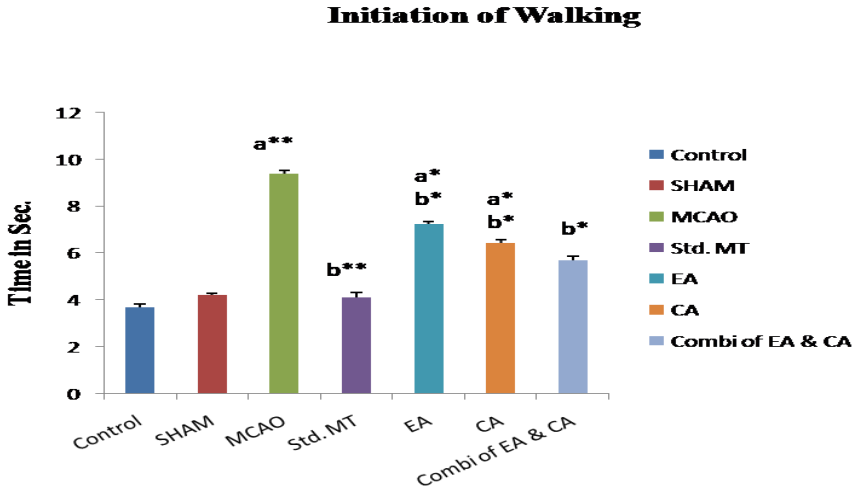

Figure 6: Effect of combination of *Eclipta alba* and *Centella asiatica* on initiation of walking in middle cerebral artery occlusion induced brain stroke in experimental animal ratss. All values are expressed as Mean ± SEM. ***p = 0.001, **p<0.01. a*vs.* SHAM, b *vs.*MCAO.

Effect of *Eclipta alba (Eclipta alba)* extract, *Centella asiatica (Centella asiatica)* and the combination of (*Eclipta alba and Centella asiatica*) on sensory motor function via hanging performance in middle cerebral artery occlusion induced brain stroke in experimental rats.

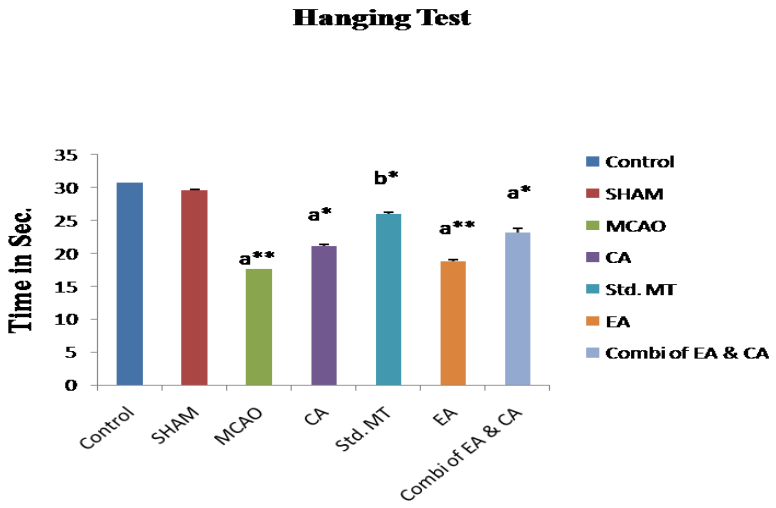

Figure 7: Effect of combination of *Eclipta alba* and *Centella asiatica* in Hanging test in middle cerebral artery occlusion induced brain stroke in experimental animal rats. All values are expressed as Mean ± SEM. ***p = 0.001, **p<0.01. a*vs.* SHAM, b *vs.*MCAO.

Effect of *Eclipta alba (Eclipta alba)* extract, *Centella asiatica (Centella asiatica)* and the combination of (*Eclipta alba and Centella asiatica*) on sensory motor function via locomor activity in middle cerebral artery occlusion induced brain stroke in experimental rats.

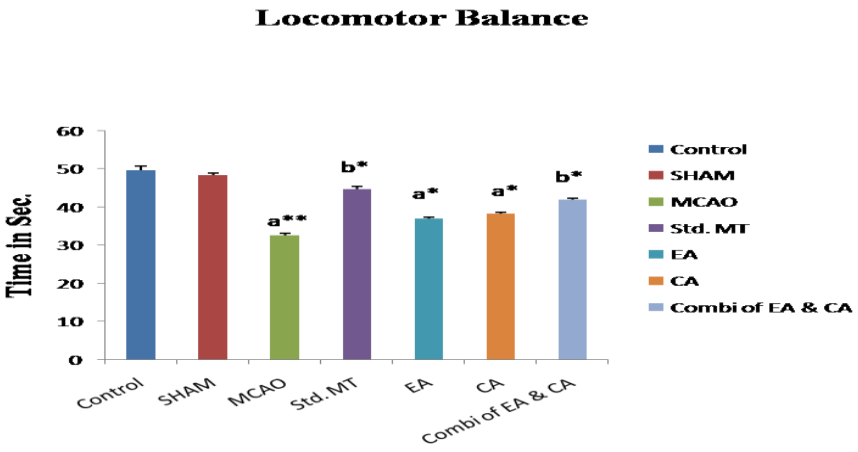

Figure 8. Effect of combination of *Eclipta alba* and *Centella asiatica* on Latency to fall-pole (Locomotor balance) in middle cerebral artery occlusion induced brain stroke in experimental animal rats.

4. Statistical analysis Statistical data was analyzed using Sigma Stat 3.5. One way ANOVA was used to compare between the means of seven groups and tukey tests were applied to determine statistical difference between each of those groups. P value <0.05 was considered as significant.

5. DISCUSSION

Animal models of focal cerebral ischemia in which MCAO was used to reproduce the pattern of ischemic brain damage observed in many human ischemic stroke patients.[13] It is a focal neurologic deficit which is caused by a change in cerebral circulation. In our own preliminary experiments in which uncoated sutures were used, the success rate was also rather low. Thus, we adopted the method of (Longa, Weinstein et al. 1989). Damage to cell structures was mediated by reactive oxygen species, including membranes, lipids, proteins, and DNA. All among the area it was observed the cerebral vasculature was a major target of oxidative stress and it played a critical role in the pathogenesis of ischaemic brain injury. The effect of middle cerebral artery occlusion model (MCAO) in animal mimicked one of the most common types of ischemic stroke in patients. According to the variable in reperfusion times, the model offers different grades of damage ranging from transient ischemic attack (TIA) to large infarcts including major parts of the ischemic hemisphere. This allows us to study different pathophysiological mechanisms after stroke. MCAO surgery was performed in a short time period and produces highly reproducible lesions. Furthermore, due to variances in cerebral vascular anatomy, different mouse strains show different outcomes.[14] The body temperature also affects damage of neurons, in hypothermia conditions it leads to smaller lesions and in hyperthermia conditions it more to severe deficits.[15] So temperature control and maintenance was highly relevant in this model.[16] The choice of the anaesthetic was also highly important, as some might have neuroprotective effects, and/or be vasodilators, as for example Isoflurane.[17] Consequently, exposure to anaesthesia should be as short as possible and standardized. We exclude animals which had undergone surgery for longer than 15 min. Infarction is the death of tissue (necrosis) caused by a local lack of oxygen, due to an obstruction of the blood supply to the tissues. The resulting lesion is referred to as an infarct. In the results of brain sections it showed infract volume in the brain of MCAO treated animal group was similar to stroke patients. Interestingly, when EA and CA treated group animals followed by MCAO surgery were individually showed a significant decrease in infract volume and but the combination of both herbs was much nearer to the effect of the standard drug MT. Standard drug melatonin

shows very less infract or no infract area. Excitotoxicity parameters were also disturbs in stroke, in MCAO treated group glutathione (GSH) level was decreased and malondialdehyde (MDA) level was increased in rat brain but in MCAO with *Eclipta alba* and *Centella asiatica* extract pretreated group GSH level was significantly increased and MDA level was significantly decrease in compare to MCAO surgery group, In melatonin with MCAO treated group GSH level was increased and reach nearer to the control and SHAM treated group. Effect of the combination of *Centella asiatica* and *Eclipta alba* in excitotoxicity parameters was about to standard drug (MT). The results of sensory motor function (initiation of walking, beam balance, hanging wire, locomotor balance and coordination) showed impaired motor functions in MCAO treated group animals. It was similar to the stroke patients motor function tests. There was shown significantly improvement in sensory motor functions (improves latency to move, improves balance in beam, improvements in latency to fall, improve locomotor balance and muscles cordination) in the combination of *Eclipta alba* and *Centella asiatica* group when compared to the MCAO treated group. And also the roots and leaves of Eclipta alba and entire plant of *Centella asiatica* are effective in wound healing.[18,19]

6. Conflict of interest

I declare that this article content has no conflict of interest.

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