

**Original Research Article****Role of *Rauwolfia serpentina* in stroke induced experimental dementia**

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ABSTRACT

Objective: To investigate the Role of *Rauwolfia serpentina* in stroke induced experimental dementia.

Methods: Methanolic root extract of *Rauwolfia serpentina*(RS) at a dose of 10mg/kg,20mg/kg and 40mg/kg;p.o. was studied against global cerebral ischemia induced dementia by occluding both common carotid arteries. RS at a dose of 10mg/kg, 20mg/kg and 40mg/kg;p.o. were administered for 14 days before and 7 days after both common carotid arteries occlusion(BCCAO) and were continued during behavioral testing i.e. Morris water maze test and elevated plus maze test. At the end of all experiment mice brain were removed and TBARS level, SOD level and infarct size were determined.

Results : Occlusion of both common carotid arteries significantly increased escape latency time(ELT) during acquisition trial and decrease, time spent in target quadrant on morris water maze, an increase in escape latency time was also observed on elevated plus maze when measured after 7 days of occlusion. Furthermore, TBARS levels, SOD levels and infarct size were also increased when measured after 12 days of occlusion. Administration of RS specially at a dose of 20mg/kg and 40mg/kg;p.o. significantly attenuated these alteration and show neuroprotective effect against oxidative stress, infarct size and learning and memory impairment. **Conclusions:** These finding suggest that *Rauwolfia serpentina* may have a promising role as a prophylactic drug in stroke induced experimental dementia due to its neuroprotective effect. The neuroprotective effect of *Rauwolfia serpentina* can be attributed to the phenolic compound like phenolic acid, flavanoids and tannins present in the plant.

Introduction

Stroke is the second leading cause of death and disability[1,2] worldwide after heart disease and cancer and is the major cause of morbidity, particularly in the middle aged and elderly population[3,4]. Stroke is a serious neurological disease[1,2]. Stroke, according to the American Heart Association (AHA) definition, is a sudden loss of brain function due to disturbance in the cerebral blood supply with symptoms lasting at least 24 hours or leading to death with vascular background as its only cause. *Stroke* is defined as an "acute neurologic dysfunction of vascular origin with sudden (within seconds) or at least rapid (within hours) occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain[5]. Stroke can result in both focal and global neurological impairments including asthenia, severe headaches, hemiparesis, and blackouts. Stroke can be caused either by rupture of a blood vessel or aneurysm (hemorrhagic stroke, HS), or by thrombosis or embolisms (ischemic stroke, IS)[4,6].

Ischemic stroke occurs when the blood supply to a part of the brain is suddenly interrupted by occlusion[7-9]. Ischemic cerebrovascular disease is mainly caused by thrombosis, embolism and focal hypoperfusion, all of which can lead to a reduction or an interruption in cerebral blood flow (CBF) that affect neurological function due to deprivation of the glucose and oxygen[1,5,10,11]. Within seconds to minutes after the loss of blood flow to a region of the brain, the ischemic cascade is rapidly initiated [12].

Oxidative stress such as generation of damaging reactive oxygen species has been proven to play a key role in pathogenesis of cerebral ischemia[13]. Several oxygen free radicals (oxidants) and their derivatives are generated after stroke, including superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$). $O_2^{\cdot-}$ are formed within the mitochondria when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron[14]. In the ischaemic cell, O_2 levels are depleted before glucose, favouring a switch to the glycolytic pathway of anaerobic ATP production. This results in lactic acid and H^+ production within the mitochondria and the subsequent reversal of the H^+

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uniporter on the mitochondrial membrane which causes excess cytosolic H^+ accumulation and acidosis[15]. Acidosis contributes to oxidative stress by providing H^+ for the conversion of $\bullet O_2^-$ into H_2O_2 or the more reactive hydroxyl radical ($\bullet OH$). The reperfusion after ischemia leads to production of superoxide and hydroxyl radicals which overwhelms endogenous scavenging mechanism. Superoxide can cause oxidative damage of iron/sulfur clusters of aconitase, an important enzyme in the tricarboxylic acid cycle[9,16]. Hydroxyl radical, peroxyxynitrite and peroxyxynitrite-derived products (hydroxyl radical, carbonate radical and nitrogen dioxide) all have the potential to react and damage lipids, proteins and DNA[9] in the core of brain tissue exposed to the most dramatic blood flow reduction is injured and subsequently undergoes necrotic cell death. This necrotic core is surrounded by a zone of less severely affected tissue which is rendered functionally silent by reduced blood flow but remains metabolically active. This region is known as "ischemic penumbra" and neurons in this area may undergo apoptosis after several hours or days, and therefore are potentially recoverable for some time after the onset of stroke[17].

Many studies have demonstrated that cerebral ischemia due to stroke can develop cognitive deficit and neuronal damage[13]. Stroke can be the direct or main cause of dementia, which is generally classified as multi-infarct dementia or vascular dementia[18]. Dementia syndromes diagnosed after stroke are usually considered to be vascular in origin[19]. Stroke and dementia may share common environmental factors and biological bases, the vascular lesion in the brain, white matter changes, cascade of neurodegenerative process i.e. free radicals, may have an additive effect on the development of dementia[18,20,21]. Therefore, inhibition of production of these reactive oxygen species with pharmacological agents have been found to limit the brain damage causes by stroke[22].

The lack of effective and widely applicable pharmacological treatment for stroke patients may explain a growing interest in traditional medicines, for which extensive observational and experience has accumulated over the past thousand years[23]. Accumulated evidences indicate that free radicals are involve in the pathophysiology of various diseases such as diabetes, cancer, neurodegenerative and cardiovascular diseases. Plants containing high content of antioxidant phytochemicals can prevent these diseases[24]. Increasing evidence supports the hypothesis that plant polyphenol provide protection against neurodegenerative changes associated with cerebral ischemia[25]. *Rauwolfia serpentina* belonging to the family Apocynaceae, is an important medicinal plant in the pharmaceutical world due to the presence of its immense therapeutic properties. The plant is known for curing various disorders because of the presence of various phytochemical compounds or secondary metabolites like alkaloids, carbohydrates, flavonoids, glycosides, phlobatannins, phenols, resins, saponins, sterols, tannins and terpenes[26-29]. The presence of phenolic compounds in the plant indicates that this plant have antioxidative, antidiabetic, anticarcinogenic,

antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities[24,30]. Used as an expectorant and emulsifying agent[26] but it has yet not been evaluated for its antioxidant role in stroke induced experimental dementia.

It elicit antioxidant capacity due to the observed higher phenolic contents like p-hydroxybenzoic acid and p-coumaric acid(Phenolic acid), Flavanol and Proanthocyanidins(Flavanoids), Gallic acid and Digallic acid(Tannins)[24]. The methanol root extract of *Rauwolfia serpentina* provides 233mg/gm phenol[31].

Material and methods

Plant material

Dried roots of *Rauwolfia serpentina* were collected from local market of Dehradun and authenticated by the taxonomist at Systemic Botany Discipline, Botany Division, Forest Research Institute, Dehradun. The voucher specimen no.isNo.Dis/84/2015/Syst.Bot./Rev.Gen./4-5.

Preparation of extract

Dried root purchased from local market were slightly washed under running water and dried under shade. Then roots were ground into course powder using pestle and mortar. Extraction was carried out with methanol(95%) using soxhlet apparatus for 48 hrs and crude extract was dried under room temperature and stored under 10°C temp. until use.

Chemicals and drugs

Triphenyltetrazoliumchloride (TTC), Tris buffer, Thiobarbituric acid, Trichloroacetic acid, Sodium dodecyl sulphate(SDS) were purchased from Central drug House, New Delhi. Total protein estimation kit were purchased from Himgiri traders, Dehradun. Epinephrine were purchased from Sigma Aldrich. Other chemicals and solvents used were of analytical grade purchased from commercial suppliers.

Animals

Male Swiss albino mice weighing 20-25 gm were procured from animal house of SGRRTS, Dehradun. During the experiment mice were acclimatized in the animal house facility of department and housed in polypropylene cages with hask bedding(renewed every 48 h),under 12:12 h light-dark cycle at 25° C ± 5° C. The mice were allowed free access to commercial pellet diet and water *ad libitum*. The experiments were performed between 10.00a.m. - 5.00p.m.

Ethical clearance

All the studies were carried out in accordance with the guidelines given by the Committee for the purpose of Control and Supervision of Experiments on Animals(CPCSEA),New Delhi(India) and the Institutional Animal Ethics Committee approved the study(Approval No.: M.Ph/IAEC/01/2014/ECC-10).

Experimental protocol

Animals are divided into 7 groups and each comprises of 6 animals

- ▶ **Group I** : Control group : normal saline (10ml/kg,p.o) was administered for 25 days.

- ▶ **Group II:** Sham control group- only surgical procedure was performed on 14 day.
- ▶ **Group III: Negative control group-** 0.05% DMSO (10ml/kg,p.o) was administered for 14 days before and for 7 days after occlusion of both common carotid arteries and were continued during acquisition and retrieval trial.
- ▶ **Group IV:** Standard group treated with Vit. E (100mg/kg,p.o.) for 14 days before and for 7 days after occlusion of both common carotid arteries and were continued during acquisition and retrieval trial.
- ▶ **Group V:** Test group treated with *Rauwolfia serpentina* (10mg/kg,p.o.) for 14 days before and for 7 days after occlusion of common carotid arteries and were continued during acquisition and retrieval trial.
- ▶ **Group VI:** Test group treated with *Rauwolfia serpentina* (20mg/kg,p.o.) for 14 days before and for 7 days after occlusion of common carotid arteries and were continued during acquisition and retrieval trial.
- ▶ **Group VII:** Test group treated with *Rauwolfia serpentina* (40mg/kg,p.o.) for 14 days before and for 7 days after occlusion of common carotid arteries and were continued during acquisition and retrieval trial.

Surgical procedure for Both common carotid arteries occlusion(BCCAO)

Mice were anaesthetized using chloral hydrate (400 mg/kg, i.p). A midline ventral incision was made in the neck to expose the right and left common carotid arteries, which were isolated from surrounding tissue and vagus nerve. A cotton thread was passed below both the carotid arteries. Global cerebral ischemia was induced by occluding the carotid arteries. After 10 min of global cerebral ischemia, reperfusion was allowed. The incision was sutured back in layers. The sutured area was cleaned with 70% ethanol and was sprayed with antiseptic dusting powder. The animals were shifted individually to their home cage and were allowed to recover overnight. During surgery, the animals were kept on a heating pad in order to maintain the body temperature, so as to avoid the effect of temperature variations on the final results.

Behavioral testing

Morris water maze[32]: The Morris water maze test was employed to assess learning and memory of the animals. The Morris water maze is a swimming –based model in which animals learn to escape a pool of water (150cm in diameter, 45cm in height, filled to depth of 30 cm with water at 28± 1°C) by the hidden platform. Then a white non-toxic dye was added to the water to make it opaque so that mice didn't able to see the platform position. Then threads were fixed at right angle to each other in the rim of the pool so that the pool was divided into four quadrants i.e.Q1, Q2, Q3 and Q4. A platform of 10 cm² was placed in the target quadrant of the pool 1 cm below surface of water. The platform position was maintained throughout the training session. Each animal was subjected to four consecutive training trails on each day with an inter-trial interval of 5 min. Each mouse was gently placed into the

water starting with a specific quadrant and facing the pool wall. The starting position was changed for each trial, while the target quadrant(Q4) remained constant. The mice were allowed 120 second to locate the submerged platform and upon finding the platform, rats were allowed to stay on the platform for 20 sec. If mice failed to reach the platform within 120 sec, they were gently guided onto the platform and allowed to remain there for 20 sec. The time taken by the mice to locate the hidden platform in the water maze is known as escape latency time(ELT). Mice were subjected to training trials(acquisition) for four consecutive days.

The platform was removed on day 5 and each mouse was allowed to explore the pool for 120 sec. Mean time spent in all four quadrants was noted. The mean time spent by the mice in the target quadrant in search of hidden platform was noted as an index of retrieval. The position of experimenter should always remain same. Care was taken to maintain the location of the water maze relative to other objects in the laboratory so that prominent visual clues would not be disturbed during the study. All the trials were completed between 10.00a.m. - 5.00p.m.

Elevated plus maze[33] : Elevated plus maze was performed by the method described by Sharma A.C. and Kulkarni S.K.1992. The apparatus consist of two open arms(16cm×5cm) and two closed arms(16cm×5cm×12cm).The arms extended from a central platform (5cm×5cm) and the maze was elevated to a height of 25cm from the floor. On the first day, each mouse was placed at one end of an open arm, facing away from the central platform. The time taken by the mouse to move from an open arm to any one of the closed arm with all its four legs in is referred as transfer latency. If within 90 sec.the mouse did not move into one of the closed arm, it was gently pushed into it and the TL was noted as 90s. The mouse was allowed to explore the maze for 10s and then was returned to its home cage. After 24 hrs. memory retention was examined. TL measured on plus maze on first day served as an index of learning and acquisition, where as TL on 2nd day served as an index of retrieval and memory.

Biochemical analysis

After the evaluation of learning and memory animal were sacrificed on 25th day by cervical decapitation under light anaesthesia. Whole brain was carefully removed from the skull and following parameter were estimated:

Assessment of Cerebral Infarct size

[34]

Brain samples were immediately sliced into uniform coronal sections of about 1mm thickness. The slices were incubated with 1% triphenyltetrazolium chloride (TTC) at 37 °C in 0.2M Tris buffer (pH7.4) for 20 min. TTC is converted to red form zone pigment by NAD and lactate dehydrogenase and thus stained the viable cells deep red. The Infarcted cells lost the enzyme as well as cofactor and thus remain unstained dull yellow. The brain slices were placed over a glass plate. A transparent plastic grid with 100 squares in 1cm² was placed

over it. The average area of each brain slice was calculated by counting the number of squares on either side. Similarly, the number of squares falling over non-stained dull yellow area was also counted. Infarcted area was expressed as a percentage of the total brain volume. Whole brain slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size was expressed as percentage of the total wet weight of the brain.

Preparation of brain homogenate: The animals were sacrificed by cervical decapitation under light anesthesia. Whole brain was carefully removed from the skull. The fresh whole brain was weighted and transferred to a glass homogenizer and homogenized in phosphate buffer (Ph=7.4). The homogenate were centrifuged at 3000 rpm for 15 min. The supernatant was collected and used for the following biochemical parameters:

Calculation

$$\text{LPO} = \frac{\text{Test O.D.} \times \text{Total Volume} \times 100}{1.56 \times 10^5 \times 10^{-9} \times \text{Sample Volume} \times \text{mg protein per ml}}$$

Unit: nM MDA / min × mg protein

Estimation of superoxide dismutase(SOD)[37]

SOD measurement was carried out on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome. The absorbance was measured at 480 nm. 1 units of SOD

Calculation

$$\text{SOD s} = \frac{C \times \text{Total Volume} \times 1000}{50 \times \text{Sample Volume} \times \text{mg Protein per ml}}$$

Unit: Units/ mg Protein

Statistical Analysis

The statistical analysis was carried out using Graph Pad Prism 5.03 software. All values will be presented as Mean ± SEM. Multiple comparisons between different groups were performed using Analysis of Variance (ANOVA) followed by Tukey's test.

Results

Effect of global cerebral ischemia and Vit.E on acquisition and retrieval of memory using morris water maze

Within control group there was a significant (P<0.001) decrease in ELT when day1 was compared with day 2, day 3 and day 4. Within sham control group there was a significant (

Estimation of total protein in the brain[35]

Protein values were estimated to express the biochemical parameters in terms of per mg protein. The quantitative measurement of the total protein was determined by Lowry's method.

Measurement of lipid peroxidation(LPO)[36]

The measurement of malondialdehyde(MDA), which is most abundant of lipid peroxidation products, is a convenient and sensitive method for quantitative estimation of lipid peroxide concentration in many types of samples including biological tissues. MDA level were estimated spectrophotometrically by measuring thiobarbituric acid reactive substances(TBARS) at 532nm. The results were expressed as nM of MDA/mg of wet tissue using molar extinction co-efficient of the chromophore $1.56 \times 10^5 \text{ mmol}^{-1} \text{ cm}^{-1}$ as 99% of TBARS is MDA.

produce approximately 50% of inhibition of auto-oxidation of adrenaline. The results are expressed as unit (U) of SOD activity per mg of tissue.

P<0.001) decrease in ELT when day1 was compared with day 2, day 3 and day 4. Negative control mice showed a significant (P<0.001) increase in ELT when compared with the control group in same day (fig.3.1), indicating impairment of acquisition. Negative control mice also showed a significant (P<0.001) decrease in time spent in target quadrant when compared with the control group (fig.3.3.), reflecting impairment of retrieval memory. Treatment with Vit.E attenuated the cerebral ischemia induced increase in ELT on day1 (P<0.01), day2, day3, day4 (P<0.001) (fig.3.1.) and decrease in time spent in target quadrant (P<0.001) (fig.3.3.) as compared to negative control group, indicating reversal of cerebral ischemia induced learning and memory deficit.

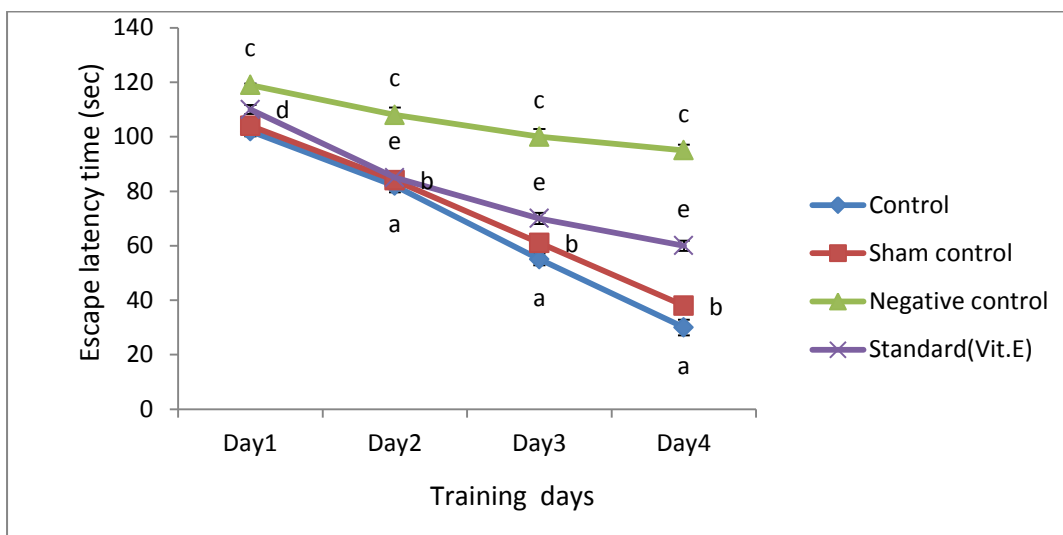


Fig. 3.1. Effect of global cerebral ischemia and Vit.E on escape latency time(ELT) during acquisition trials in mice on morris water- maze. Values are express as mean± SEM (n=6) a= P<0.001 Vs. ELT of day 1 within control; b= P<0.001 Vs. ELT of day 1 within sham control; c=P<0.001 vs. ELT in control on same day; d= P <0.01 Vs ELT in negative control on same day.

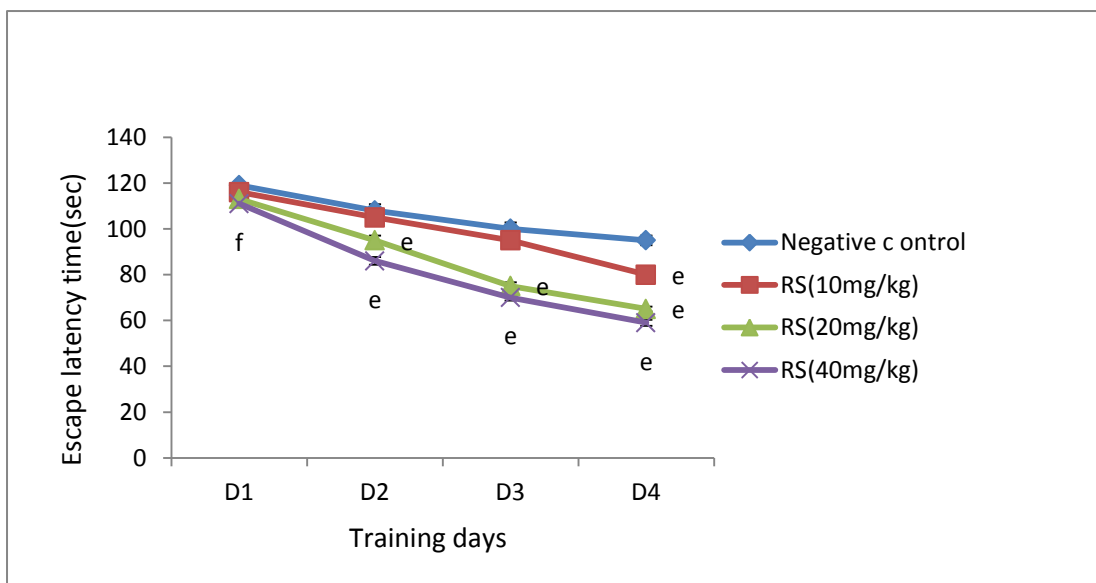


Fig.3.2. Effect of global cerebral ischemia and *Rauwolfia serpentina*(RS) on escape latency time(ELT) during acquisition trials in mice on morris water maze Values are express as mean± SEM (n=6); e= P <0.001 vs. ELT in negative control on same day; f= P <0.05 Vs ELT in negative control on same day.

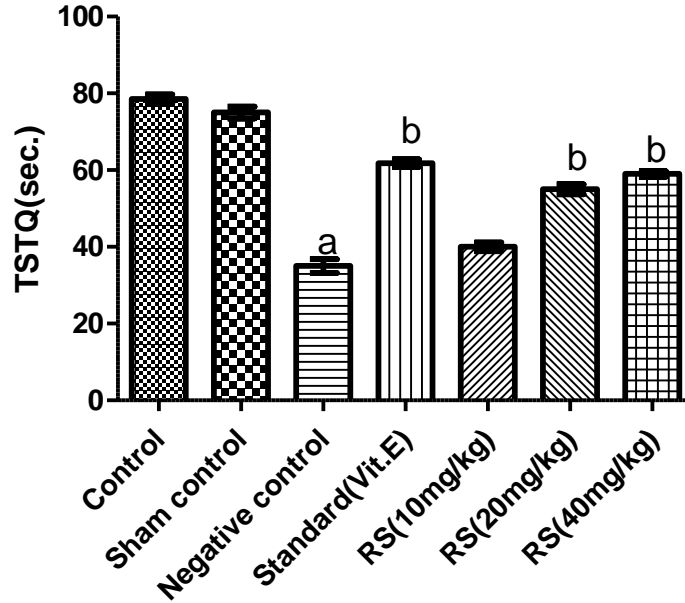


Fig. 3.3. Effect of RS on time spent in target quadrant on morris water maze. Values are express as mean±SEM; p<0.001 was considered as statistically significant;a=p<0.001 Vs control; b= p<0.001 Vs negative control.

Effect of global cerebral ischemia and *Rauwolfia serpentina*(RS) on acquisition and retrieval of memory using morris water maze

Administration of *Rauwolfia serpentina* at dose 10mg/kg;p.o. did not show any significant change in ELT on day1,day2 and day3 and time spent in target quadrant on day5 when compared with negative control group. However, RS (10mg/kg) show significant(P<0.001)(fig.3.2) decrease in ELT on day 4 when compared with negative control group. *Rauwolfia serpentina* at dose 20mg/kg;p.o. significantly(P<0.001) attenuated the cerebral ischemia

induced rise in ELT(fig.3.2) and decrease time spent in target quadrant(fig.3.3) except on day 1 as compared to negative control group,indicating reversal of cerebral ischemia induced learning and memory deficit. R.serpentina at dose of 40mg/kg; p.o. show significant(P<0.001)(fig.3.2) decrease in ELT(on day2, 3 and 4) during acquisition trials and significant(P<0.001)(fig.3.3)increase time spent in target quadrant on day 5 when compared with negative control group. It also show significant(P<0.05)(fig.3.2) decrease in ELT(day 1) when compared with negative control group.

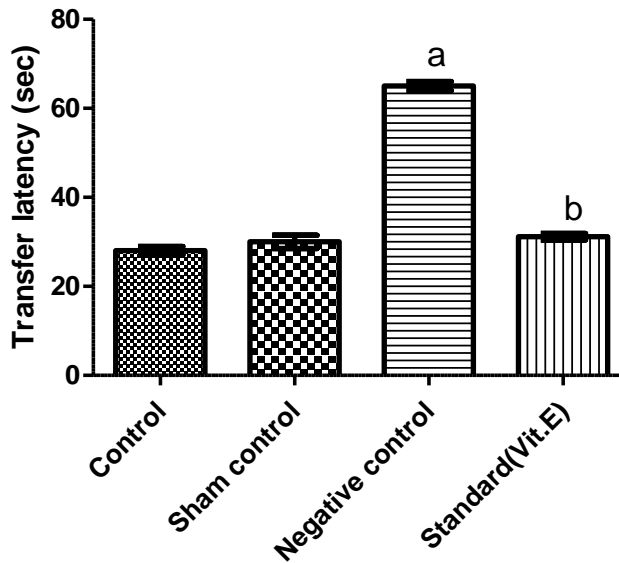


Fig.3.4. Effect of global cerebral ischemia and Vit.E on Transfer latency(TL) using Elevated plus maze Values are express as mean±SEM ;p<0.001 was considered as statistically significant.;a=p<0.001 Vs control; b= p<0.001 Vs negative control.

Effect of global cerebral ischemia and Vit.E on Transfer latency(TL) using Elevated plus maze task

Transfer latency(TL) of negative control group significantly($p < 0.001$)(fig.3.4) increase as compared to control, indicating impairment in learning and memory.

Treatment with Vit.E(100mg/kg) significantly ($p < 0.001$)(fig.3.4) lowered the TL in cerebral ischemia induced mice, indicating improvement in learning and memory.

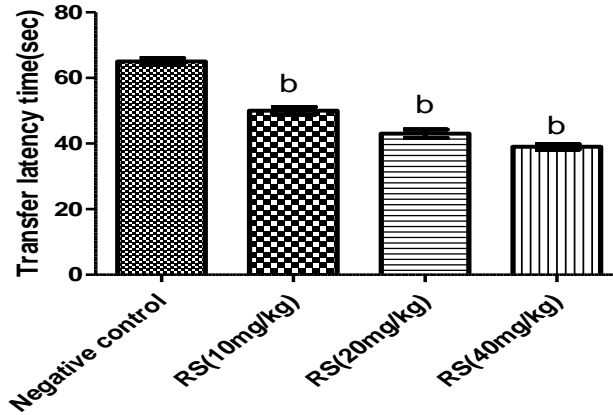


Fig. 3.5. Effect of global cerebral ischemia and *Rauwolfia serpentina*(RS) on Transfer latency(TL) using Elevated plus maze
 Values are express as mean±SEM ; $p < 0.001$ was considered as statistically significant.; $b = p < 0.001$ Vs negative control.

Effect of global cerebral ischemia and *Rauwolfia serpentina*(RS) on Transfer latency(TL) using Elevated plus maze task

Administration of RS(10mg/kg,20mg/kg and 40mg/kg;p.o.) significantly($p < 0.001$) lowered the TL in cerebral ischemia induced mice, indicating improvement in learning and memory.(fig.3.5)

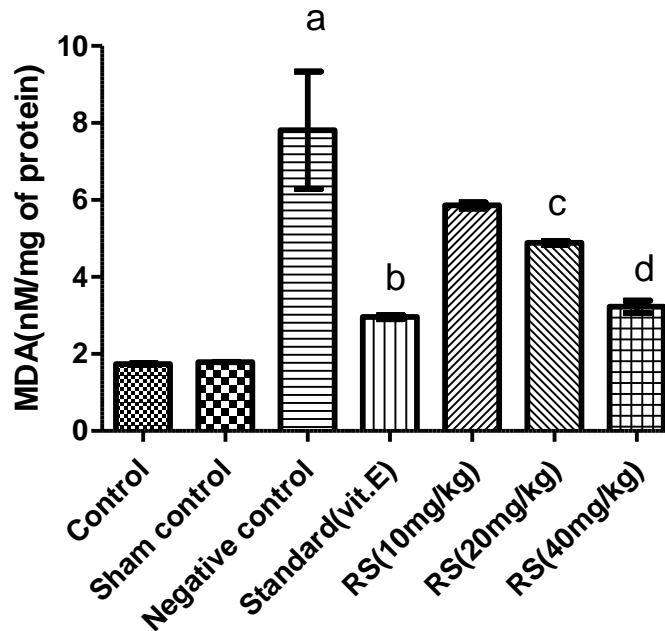


Fig.3.6 Effect of global cerebral ischemia and *Rauwolfia serpentina*(RS) in brain thiobarbituric acid reactive substances(TBARS) level. Each group represent as mean ± SEM.a= $p < 0.001$ vs control group; b= $p < 0.001$ vs negative; c= $p < 0.05$ vs negative; d= $p < 0.01$ vs negative group

Effect of stroke and *Rauwolfia serpentina* (RS) in brain thiobarbituric acid reactive substances(TBARS) level

Negative control group produced a statistical significant($p<0.001$) increase in brain oxidative stress levels, as determined by increased thiobarbituric acid reactive species(TBARS) levels, when compared to control group. Administration of Vit.E produced significant ($p<0.001$)

reduction on cerebral ischemia induced rise in TBARS levels as compared to negative group. Administration of RS (20mg/kg and 40mg/kg) significantly ($p<0.05$ and $p<0.01$) reduced TBARS levels as compared to negative group. RS at the dose of 10mg/kg did not show any significant result as compared to negative control. (fig.3.6)

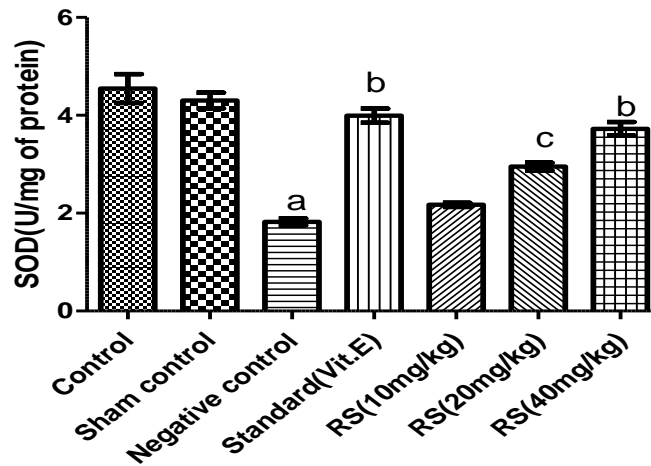


Fig.3.7. Effect of global cerebral ischemia and *Rauwolfia serpentina*(RS) in brain SOD level. Each group represent as mean \pm SEM. a= $p<0.001$ Vs control group; b= $p<0.001$ Vs negative group; c= $p<0.01$ Vs negative group.

Effect of stroke and *Rauwolfia serpentina*(RS) in brain SOD level

Negative control group produced a statistical significant($p<0.001$) increase in brain oxidative stress levels, as determined by decreased superoxide dismutase(SOD) levels, as compared to control group. Administration of Vit.E

produced significant ($p<0.001$) increase on cerebral ischemia induced fall in SOD levels as compared to negative group. Administration of RS (20mg/kg and 40mg/kg) significantly ($p<0.01$ and $p<0.001$) increased SOD levels as compared to negative group. RS at the dose of 10mg/kg did not show any significant result as compared to negative control. (fig.3.7)

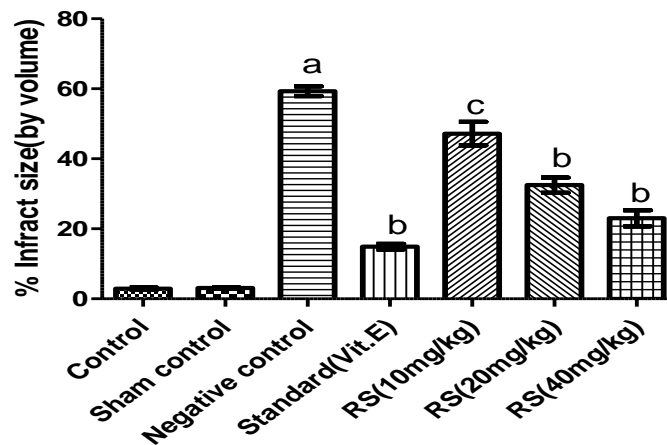


Fig.3.8. Effect of *Rauwolfia serpentina*(RS) on global cerebral ischemia induced cerebral infarct size in mice by volume method. Each group represent as mean \pm SEM. a= $p<0.001$ Vs control group; b= $p<0.001$ Vs negative group; c= $p<0.01$ Vs negative group.

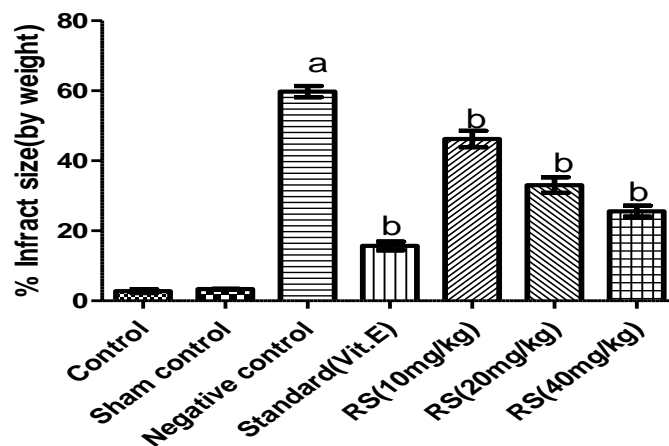


Fig.3.9. Effect of *Rauwolfia serpentina*(RS) on global cerebral ischemia induced cerebral infarct size in mice by weight method. Each group represent as mean \pm SEM. a= $p < 0.001$ Vs control; b= $p < 0.001$ Vs negative control

Effect of *Rauwolfia serpentina* on cerebral infarct size

Global cerebral ischemia of 10 min followed by reperfusion for 12 days produced a significant ($P < 0.001$) increase in the cerebral infarct size in negative control group compared to the control and sham group when measured by both volume and weight methods. When we compared the control and sham group there is no significance difference in cerebral infarct size when measured by both volume and weight methods. *R. serpentina* (10mg/kg, 20mg/kg and 40mg/kg; p.o.) extract and Vit. E administered 14 days prior and 12 days after occlusion of both common carotid arteries significantly ($P < 0.001$) decreased the cerebral infarct size when compared with negative control group (fig.3.8 and fig.3.9) that was measured by both volume and weight method.

Discussion

In the present study an attempt was made to evaluate the effect of *Rauwolfia serpentina* on stroke induced experimental dementia. According to the *Global Burden of Disease Study 2010* in the last decade stroke became the third-most-common global cause of disability-adjusted life years (DALYs), second only to ischemic heart disease. Increase in vascular risk factors such as high blood pressure, tobacco smoking, chronic alcoholism, and poor diet seems to be responsible for this increase [38].

It is well known that stroke is a sudden loss of brain function due to disturbance in the cerebral blood supply with symptoms lasting at least 24 hours or leading to death with vascular background as its only cause. *Stroke* is defined as an "acute neurologic dysfunction of vascular origin with sudden (within seconds) or at least rapid (within hours) occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain [5].

Stroke can result in both focal and global neurological impairments including asthenia, severe headaches, hemiparesis, and blackouts. Stroke can be caused either by rupture of a blood vessel or aneurysm (hemorrhagic stroke, HS), or by thrombosis or embolisms (ischemic stroke, IS) [4,6].

Oxidative stress such as generation of damaging reactive oxygen species has been proven to play a key role in pathogenesis of cerebral ischemia [13]. In ischaemic state, O_2 levels are depleted in brain cells before glucose, favouring a switch to the glycolytic pathway of anaerobic ATP production. This results in lactic acid and H^+ production within the mitochondria and the subsequent reversal of the H^+ uniporter on the mitochondrial membrane which causes excess cytosolic H^+ accumulation and acidosis [15]. Acidosis contributes to oxidative stress by providing H^+ for the conversion of $\bullet O_2^-$ into H_2O_2 or the more reactive hydroxyl radical ($\bullet OH$). The reperfusion after ischaemia leads to production of superoxide and hydroxyl radicals which overwhelms endogenous scavenging mechanism. Superoxide can cause oxidative damage of iron/sulfur clusters of aconitase, an important enzyme in the tricarboxylic acid cycle [9,16].

Overproduction of ROS during ischemia /reperfusion can damage lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis [25]. The lack of effective and widely applicable pharmacological treatment for stroke patients may explain a growing interest in traditional medicines, for which extensive observational and experience has accumulated over the past thousand years. According to the WHO report (2003), traditional medicine is very popular in all developing countries, and its use is rapidly increasing in industrialized countries [23].

Many effective components extracted from traditional herbs have been demonstrated to show neuroprotection against ischemic brain injury in experimental studies [39].

Accumulated evidences indicate that free radicals are involved in the pathophysiology of various diseases such as diabetes, cancer, neurodegenerative and cardiovascular diseases. Plants containing high content of antioxidant phytochemicals can prevent these diseases [24]. Increasing evidence supports the hypothesis that plant polyphenol provide protection against neurodegenerative changes associated with cerebral ischemia [25]. *Rauwolfia serpentina* elicit antioxidant capacity due to the observed higher phenolic contents like p-hydroxybenzoic acid and p-coumaric acid (Phenolic acid),

Flavanol and Proanthocyanidins (Flavanoids), Gallic acid and Digallic acid (Tannins) [24]. The methanol root extract of *Rauwolfia serpentina* provides 233mg/gm phenol [31]. The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen. A highly significant, positive correlation was found between antioxidant capacity and phenolic content, indicating that phenolic compounds are a major contributor to antioxidant activity [31].

Many studies have demonstrated that cerebral ischemia can develop cognitive deficit and neuronal damage [13]. Both common carotid artery occlusion (for 10-20min) is the most common and easy method used to induce cerebral ischemia and therefore produce impairment of learning and memory, with acute neuronal death in hippocampi, particularly CA1 cells, apoptotic death of oligodendrocytes in cortex and thalamus 3-7 days later [40]. Therefore global cerebral ischemia was induced by occluding the carotid arteries. After 10 min of global cerebral ischemia, reperfusion was allowed. It was indicated by the formation of cerebral infarction, increase in the malondialdehyde level, and decrease in the SOD level.

It is well known that the hippocampus is the brain area that play an important role in spatial memory [41]. Therefore, we determined the cognitive performance of the mice using the Morris water maze test which is the best tool to determine spatial learning memory in rodents [42]. The Morris water maze is a hippocampus-dependent memory task that has been used to assess cognitive deficits in the ischemic brain. In the present study the hidden platform trial measured acquisition and the time spent in target quadrant in search of missing platform measures retention of memory. The results exhibited that both learning capacity and memory were gradually impaired in the mice with global cerebral ischemia [43]. In this test a significant decrease in ELT on day 2 to day 4 of the acquisition trials for control animals denoted acquisition of memory and an increase in TSTQ in search of missing platform during the retrieval trial conducted on day 5 indicated retrieval of memory. Negative control mice showed a significant increase in ELT when compared with the control group on same day, indicating impairment of acquisition. Negative control mice also showed a significant decrease in time spent in target quadrant when compared with the control group, reflecting impairment of retrieval memory. Treatment with Vit.E attenuated the cerebral ischemia induced increase in ELT and decrease in time spent in target quadrant as compared to negative control group, indicating reversal of cerebral ischemia induced learning and memory deficit. *R. serpentina* at dose of 20 and 40mg/kg;p.o. show significant decrease in ELT and increase in time spent in target quadrant, when compare with negative control group. Administration of *Rauwolfia serpentina* at dose 10mg/kg;p.o. do not show significant change in ELT and time spent in target quadrant except on day 4. Results shows that RS at a dose of 20 and 40mg/kg;p.o significantly attenuated cerebral ischemia induced alterations suggesting its positive role in learning and memory. Learning and memory performance was also measured in elevated plus maze test, a widely used behavioural

animal model of assessment of memory. The result from EPM further substantiate the finding of MWM test as RS treatment reduced the increased transfer latency in cerebral ischemic mice.

Excessive generation of reactive oxygen species (ROS) result in the lipid peroxidation of the membrane and subsequent damage is reflected by accumulation of MDA, a neurotoxic by-product of lipid peroxidation [44]. Concentration of MDA were assayed as an index of membrane oxidative damage. Therefore in the present study MDA was estimated using TBARS assay to estimate extent of ROS formation. Cerebral ischemia induced mice showed statistical significant increase in brain oxidative stress levels, as determined by increased thiobarbituric acid reactive species (TBARS) levels, as compared to control group. Administration of RS (20mg/kg and 40mg/kg) significantly reduced TBARS levels as compared to negative group. RS at the dose of 10mg/kg did not show any significant result as compared to negative control.

Clinical research finding also indicate reduced defense involved in pathophysiology of vascular dementia [45]. Therefore we examined the antioxidant enzyme level like SOD, which serve as oxidative indices in various brain regions. RS (20mg/kg and 40mg/kg) was found to elevate the activity of major oxygen radical species metabolizing enzyme i.e. SOD in brain of ischemic mice which show that RS possess antioxidant activity against hypoperfusion induced oxidative stress. RS at the dose of 10mg/kg did not show any significant result as compared to negative control. From this observation it is further confirm that RS methanolic extract contain phenolic compounds which by their antioxidant property show protective effect. The possible explanation for this, Phenolic compounds (Phenolic acids) inhibit lipid peroxidation by trapping the lipid alkoxyl radical they also stimulate enzyme with recognized antioxidant activity, such as superoxide dismutase (SOD) [46].

In evaluations of the effect of drug on learning and memory an important aspect is potential neuroprotective effect of drug on ischemic cells [47]. Global cerebral ischemia of 10 min produced a significant increase in the cerebral infarct size in negative control group compared to the control and sham group. These alteration were alleviated by administration of *R. serpentina* (10mg/kg, 20mg/kg and 40mg/kg;p.o.) extract 14 days prior and 12 days after occlusion of both common carotid arteries when measured by both volume and weight method.

Attenuation of reactive morphological changes as well as behavioral deficits by *R. serpentina* methanolic extract indicate that it reduces neuronal insult in the setting of cerebral ischemia caused by occlusion of both common carotid arteries.

The present finding suggest that the protective effect of methanolic root extract of *R. serpentina* on cognitive deficits and structural changes is associated with its antioxidant activity.

Conclusion

On the bases of above results and discussion we can conclude the following:

1. Occlusion of both common carotid arteries for 10 min increased escape latency time(ELT) during acquisition trial and decrease, time spent in target quadrant on morris water maze, an increase in escape latency time was also observed on elevated plus maze when measured after 7 days of occlusion. Furthermore, TBARS levels ,SOD levels and infarct size were also increased when measured after 12 days of occlusion.
2. Administration of methanolic root extract of *Rauwolfia serpentina* at a dose of 20mg/kg and 40mg/kg significantly attenuated cerebral ischemia induced these alterations and show neuroprotective effect against oxidative stress, infarct size and learning and memory impairment. At a dose of 10mg/kg *Rauwolfia serpentina* show significant neuroprotective effects on infarct size.
3. The neuroprotective effect of *Rauwolfia serpentina* can be attributed to the phenolic compound like phenolic acid, flavanoids and tannins present in the plant.
4. These finding suggest that *Rauwolfia serpentina* may have a promising role as a prophylactic drug in stroke induced experimental dementia due to its neuroprotective effect.
5. The study supports an important concept that onset of neurodegenerative disease may be delayed or mitigated with use of dietary anti-oxidants that protect against oxidative stress and neurodegeneration.

Conflict of interest statement

We declare that we have no conflict of interest.

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