1. Introduction

World Health Organization defined stroke as a "neurological deficit of cerebrovascular origin that persists beyond 24 hours or is interrupted by death within 24 hours". Stroke, also known as cerebrovascular accident (CVA), is caused by rapid interruption of blood supply to the brain by the major cerebral arteries leading to impairment of central nervous system (CNS) functions (Deb et al. 2010). The main outcomes of stroke are sudden death or adult disability because of functional impairments in the patients, and it is also a predisposing factor for epilepsy and depression. Stroke may be ischemic or haemorrhagic. Ischemic stroke alone accounts for 80% of the stroke. Inadequate blood supply to the brain leads to ischemic stroke (Paliwal et al. 2018). Based on the region of the brain affected, ischemic stroke is of two types: global ischemic stroke and focal ischemic stroke. In global ischemic stroke, the blood supply of the entire brain was significantly reduced. However in focal ischemic stroke, the blood supply was reduced in a particular region of the brain blood vessel. Loss of consciousness, impaired voice, blurred vision, and numbness are the principal symptoms of cerebral ischemia. Due to the suppression of the blood flow during the ischemic condition in the brain, the level of the ATP was significantly reduced for a short period of time. That leads to enormous cell death in susceptible regions. Therefore, cerebral blood flow is one of the crucial factors for regular functioning of the brain. The damaged region of the ischemic brain which cannot be restored is known as the ischemic core region; however we can restore the region surrounding the core known as the penumbra if treatment is available at the right time. If we are able to recover cerebral blood flow during cerebral ischemia, it limits the nerve cell death in the penumbra region. Therapeutic strategy for ischemic brain injury involves reperfusion and neuroprotection. Usually to restore blood flow in cerebral ischemia,

antithrombotic drug was administered as soon as possible. The antithrombotic drugs are of three types based on their mechanism of action. Thrombolytic drugs (recombinant tissue plasminogen activator, streptokinase, etc.) break down an already formed clot. Antiplatelet drugs (aspirin, clopidogrel, etc.) prevent the aggregation of the platelet and limit the formation of a secondary clot. Anticoagulant drugs (heparin, warfarin, dabigatran, etc.) prevent the coagulation of the blood and prevent secondary clot formation. The return of blood flow in the ischemic region leads to a secondary injury known as reperfusion injury (Fitridge et al. 2011). To protect against reperfusion injury and secure the penumbral region from further damage, neuroprotection is another therapeutic approach (Paliwal et al. 2018). However, there is uncertainty for the use of recognized neuroprotective agents in clinical studies.

1.1 Causes of Cerebral Stroke

Several risk factors are involved in the etiology of stroke. Age, gender, ethnicity, and heredity have been identified as non-modifiable risk factors for stroke. Some of the disease conditions like hypertension, diabetes mellitus, atrial fibrillation, carotid stenosis are categorized as major risk factor for the incidence of stroke in human. Apart from this, lifestyle factors which include cigarette smoking, alcohol, drug abuse, obesity, diet and physical inactivity are also associated with increased stroke risk (Palomeras et al. 2010).

1.2 Epidemiology

Stroke is one of the leading causes of death worldwide. Every two second, someone in the world suffers from a stroke and every six seconds, someone dies of a stroke. Globally, Russia and China have maximum cases of stroke (Krishnamurthi et al. 2010). However, if we talk about its incidence then according to India stroke factsheet updated in 2012, the estimated

adjusted prevalence rate is 84-262/100,000 in rural and 334-424/100,000 in urban areas (Pandian et al. 2013). Among the various states Kolkata being the highest in fatality rate 42%. Stroke rehabilitation services are not adequately developed and available in private hospitals. It has been reported that in India 0.6 million people died due to stroke in the year 2000 and it is expected to increase to 0.95 million in 2020 (Ezzati et al. 2003).

1.3 Clinical diagnosis

In stroke time is a brain. As every minute 1.9 million neuron lost irreversibly in stroke. Therefore, we have to recognize the stroke very fast in order to prevent the neuron damage. Thus an acronym is given for the symptom of stroke i.e. FAST.

F: Face drooping

A: Arm numbness

S: Speech abnormality

T: Time to call ambulance

In order to diagnose the stroke clinically, neuroimaging is essential. To evaluate the acute ischemic stroke, computed tomography (CT) and magnetic resonance imaging (MRI) offer excellent tools (Muir et al. 2006). The main objectives of stroke imaging are to rule out hemorrhage from stroke. Based on the extension of established infarct and the arterial occlusion site, the best candidates for intra veinus or intra-arterial treatments are selected. Therefore, computed tomography (CT) and magnetic resonance (MR) imaging have been extensively assessed (Wintermark et al. 2009; Kohrmann et al. 2009).

Cerebral ischemia is a medical emergencies and the treatment decision which is conservative approach i.e IV thrombolysis and/or mechanical revascularization is based on the time

window and two major imaging features: parenchymal lesion and arterial occlusion site. Parenchymal imaging establishing the diagnosis and extent of ischemia and vascular imaging determining the arterial occlusion location, by CT or MRI, are mandatory for the acute ischemic stroke evaluation. Further, information regarding the collateral flow, penumbra and core extension may have added value for individual treatment decision. The imaging modality chosen for initial AIS evaluation is mostly driven by the 24 h × 7 days immediate equipment availability and by the possibility to provide the critical information required for the different possible treatments. As a result of the easily availability, CT is the most commonly worldwide used imaging technique employed for acute stroke (Wintermark et al. 2009; Köhrmann et al. 2009; Latchaw et al. 2009).

1.4 Types of cerebral ischemia

Cerebral ischemia is a neurodegenerative disorder resulting from sudden reduction of cerebral blood flow (CBF) due to occlusion of cerebral arteries or hypoperfusion for a specific time period leading to deprivation of oxygen and primary energy sources to the brain. Cerebral ischemia is further classified into two types based on the mechanism of causes and regions affected. They are focal cerebral ischemia and global cerebral ischemia.

1.4.1 Focal cerebral ischemia

Focal cerebral ischemia is caused by thrombus or embolism formation in the blood vessels thereby reducing the blood flow to specific brain regions. Thrombus or emboli formation can occlude the cerebral arteries resulting in cerebral ischemia in the affected vascular territory. Thrombosis in the blood vessels can mediate artery-artery occlusion resulting in a reduction of CBF leading to cerebral ischemia. In such cases, the brain lesions induced either by thrombus or embolism cannot be differentiated (Hossmann et al. 2012).

1.4.1.1 Thrombosis (thrombotic stroke)

Vascular obstruction mediated by thrombosis mainly occurs by atherosclerosis. Atherosclerosis can be defined as the formation of plaque inside the arteries thereby reducing the blood supply. The plaque can be formed by fat, cholesterol, and calcium-like substances and plaque can narrow and harden the arteries to reduce the oxygen-rich blood supply to the brain resulting in thrombotic ischemia. Atherosclerotic plaques can undergo the pathological changes like thrombosis, calcifications, intra-plaque hemorrhage and ulcerations. The pathological changes by the atherosclerotic plaque will initiate the disruption of the endothelium in the vascular cell wall. Disruption of endothelium will activate destructive vasoactive enzymes resulting in adherence and aggregation of platelets to the vascular cell wall. Then initiation of inflammatory responses by leukocytes present in the site leads to thrombus formation. The size, consistency, and composition of the plaques will determine thrombus formation because the intensity of endothelium disruption based on the nature of atherosclerotic plaques. Apart from atherosclerosis, the thrombotic occlusion can be caused by clot formation due to hypercoagulation, fibromuscular dysplasia, arteritis, and dissection of a vessel wall (Fuster et al. 1990).

1.4.1.2 Embolism (embolic stroke)

Embolism refers to embolization of central circulating arteries from a variety of sources. Along with clot, fibrin and pieces of atheromatous plaque, the materials like fat, air, tumor, foreign bodies will embolize into the central circulation leading to occlusion in the arteries. The emboli-induced occlusion mostly happens in superficial branches of cerebral arteries, especially in the middle cerebral artery. Embolism not only causes vascular territory occlusion alone but also has the ability to induce vasospasm in the arteries by activating

vascular irritant. The embolus lodges-induced vasospasm will occur in vascular segment and the entire arterial tree thereby reduces the central circular. Young people are more susceptible to emboli vasospasm due to more liable vessels and less atherosclerosis (Garcia et al. 1994).

1.4.2 Global cerebral ischemia

Global cerebral ischemia is caused by a drastic or complete reduction in the blood supply to entire brain regions due to hypoperfusion and it is commonly caused by cardiac arrest or hypotension. It is also referred to as hypotensive stroke because global ischemia can occur due to a profound reduction of systemic blood pressure due to some reason in the physiology system. The territories of the anterior, middle, and posterior cerebral arteries are mostly global ischemia thereby increasing the ischemic incidence in parietal-temporal-occipital junctions. Two types of injuries are most common in the global ischemic cascade, hypoperfusion and reperfusion injury (Harukuni et al. 2006).

1.4.2.1 Hypoperfusion injury

Hypoperfusion is the primary injury causing global ischemia where the neuronal injury takes place due to reduced blood perfusion to the brain tissue. This commonly happens in situations like cardiac arrest or hypotensive shock. Hypoperfusion in the brain will create demand for primary energy substrates followed by excitotoxicity, oxidative stress and inflammation leading to neuronal death.

1.4.2.2 Reperfusion injury

Reperfusion injury is a secondary insult of global ischemia. After hypoperfusion injury, the blood circulation will return into the brain tissue to fulfill the demand created by hypoperfusion, but instead of compensating the energy demand, the tissue damage will be

mediated by blood reperfusion due to induction of oxidative Stress, inflammation in the ischemic region rather than restoration of normal function. Reperfusion injury also takes place in focal ischemia after occlusion of arteries (Harukuni et al. 2006).

1.5 Animal models of focal cerebral ischemic stroke

Animal models of cerebral ischemia have been developed to mimic the ischemic events found in human stroke. Cerebral ischemic models in animal are used to understand the pathophysiology of ischemia, to develop new therapies by evaluating putative neuroprotectives agents in those models. Several ischemic models have been developed in animals based on nature of ischemia. Since 80% of the human strokes arc ischemic in origin, most of the animal models are associated with cerebral ischemia. Among the animal species rats are the most common animals used for focal cerebral ischemic models (Sicard et al. 2009). Focal ischemia is mainly induced in rat by occlusion or blockage of middle cerebral artery (MCA) territory. The reasons for using rats in the focal ischemic models include resemblance to human cerebral anatomy and physiology, the small size of the brain which enables easy analysis of brain and significant genetic homogeneity within strains (Coyle et al. 1982).

Most common rat focal ischemia models are intraluminal middle cerebral artery occlusion (MCAO) model, thromboembolic model, non-clot embolus model, photochemically induced thrombosis model and endothelin-induced MCA occlusion model (Sicard et al. 2009). On the other global ischemic animal models are developed to mimic the global ischemic conditions resulting from cardiac arrest or hypotensive conditions. Mostly mice are used for induction of global ischemia. Induction of global ischemia by occlusion of both carotid arteries is a

suitable and reproducible model (Traystman et al. 2003). MCAO models in mice are also available to evaluate the newer drugs in focal ischemic condition. Apart from this gerbils are the most common animals to induce ischemia and evaluate neuroprotective agents (Jarrott et al. 1980).

1.6 Role of platelets in focal cerebral ischemic stroke

Platelets have attained a major role in the development and progression of focal cerebral ischemia by virtue of their involvement in thromboemboli that may initiate stroke symptoms (Del et al. 1998). Platelets are one of the component of vascular system involved in the prevention of hemorrhage. After a vascular injury, platelets were activated initiating hemostatic plug formation which provides a scaffolding for coagulation activation. Since ischemic stroke accounts for approximately 80% of all strokes and results from a thrombotic or embolic occlusion of a major cerebral artery (most often middle cerebral artery, MCA) or its branches (Durukan et al. 2007), platelets activation has been an interesting target for cerebral ischemic stroke. Vascular occlusion, during ischemic stroke, resulted in CBF reduction upto 20-30% of normal blood flow. Vascular occlusion required platelet adhesion, spreading, and aggregation to reduce the CBF. The surface receptor integrin $\alpha_2\beta_1$ was involved in the binding of platelets to collagen beneath the endothelium. This binding initiated platelet activation and triggered Ca^{2+}-mediated intracellular signaling events, which lead to actin polymerization and reorganization, the formation of pseudopodia, and ultimately to cell spreading. As platelets adhered and spread, cytoplasmic organelles, α-granules and **δ** (dense)-granules consolidated within the center of the cells. With activation, both α-and δ-granules merged with the limiting membrane and released their contents into the plasma. The granular contents [which include adenosine diphosphate (ADP), β -thromboglobulin (β-TG),

platelet factor 4 (PF41), serotonin, von Willebrand factor (vWF), and fibrinogen] act to stimulate a cascade of excitatory reactions that lead to platelet aggregation (Del et al. 1998). Aggregation was initiated as exposed platelet receptors bind to circulating fibrinogen, also released from platelets to form cross-links between neighboring platelets.

1.7 Current guideline for the treatment of ischemic stroke as per American Stroke Association

Pharmacotherapy of stroke involves two strategies which are primary and secondary treatment. Primary treatments involve thrombolytic, antiplatelet and anticoagulants. Thrombolytic drugs involve intravenous recombinant tissue plasminogen activator (rt-PA) and streptokinase. The only US FDA approve drug for the treatment of stroke is rt-PA. rt-PA in a dose of 0.9 mg/kg given over one hour has been licensed in the USA since 1996 for use within 4.5 h after onset of stroke. Antiplatelet includes aspirin, clopidogrel and dipyridamole given within 48 hours of stroke. It prevents early recurrent ischemic stroke. An antithrombotic agent is a drug that reduces the formation of blood clots (thrombi). Warfarin, apixaban, dabigatran, edoxaban and rivaroxaban are examples of anticoagulants for long-term use. Secondary treatment involves neuroprotectives which are used as an adjuvant therapy. Neuroprotectives includes gingko mihuan lumbricus rubellus extract, fasudil, cerebrolysin, citicoline, kallidinogenase and edaravone.

Table 1.1: Available treatment of ischemic stroke as per American Stroke Association and their shortcomings

Condition	Recommendation	Shortcomings
Acute stroke 3-4.5hrs	rtPA(0.9mg/kg)	Administer within 3-4.5hrs Risk of intra cerebral hemorrhage Low efficacy in recanalization of larger intracranial arteries (internal carotid artery (ICA) or proximal MCA)
Acute stroke 6-16hrs	Mechanical thrombectomy	Limited access to trained neurointerventionalists, technical difficulty with the navigating wire in delicate intracranial vessels, trauma to vessels, distal embolization, vessel dissection and vasospasm leading to worsening of stroke
Acute stroke 24hrs	Aspirin (75-325mg/d) Aspirin (75-325mg/kg) plus Clopidogrel (75mg/kg)	Gastric bleeding/Ulcer Aspirin resistance i.e. inability of aspirin to reduce platelet activation and aggregation of thromboxane A2, 40% of aspirin users are resistant to aspirin, due to genetic polymorphisms of COX-1 and other genes involved in thromboxane biosynthesis (Hankey et al. 2006) Clopidogrel plus aspirin had a lower risk of major ischemic events but a higher risk of major hemorrhage at 90 days than those who received aspirin alone

1.7.1 Available treatment option of ischemic stroke and their limitation:

For eligible patients with acute ischemic stroke, intravenous alteplase (recombinant tissue plasminogen activator or tPA) is first-line therapy, provided that treatment is initiated within 4.5 hours of clearly defined symptom onset. Because the benefit of alteplase is time dependent, it is critical to treat patients as quickly as possible. Eligible patients should receive intravenous alteplase without delay even if mechanical thrombectomy is being considered (Campbell et al. 2006; Furlan et al. 2015). Mechanical thrombectomy is indicated

for patients with acute ischemic stroke due to a large artery occlusion in the anterior circulation who can be treated within 24 hours of the time last known to be well (Cohen et al. 2015; Chia et al. 2015).

Mechanical thrombectomy is type of minimally-invasive procedure in which an interventional radiologist uses specialized equipment to remove a clot from a patient's artery. Using fluoroscopy, or continuous x-ray, the doctor guides instruments through the patient's arteries to the clot, extracting the clot all at once. The radiologist starts the procedure by making a small incision in either the wrist or the abdomen, giving them to access to an artery. After making the incision, the doctor threads a catheter through the artery to the clot. Next, they insert a tiny net-like device called a stent retriever into the catheter and guide it to the blockage. They then push the stent retriever through the clot (Jadhav et al. 2018). After the stent retriever is through, it expands to the size of the artery wall. At this point, the stent retriever has captured the clot, and the doctor is able to pull it out backwards, removing the clot entirely (Campbell et al. 2006).

Time dependent treatment issues may limit the widespread clinical use of tPA and mechanical thrombectomy. First, only an estimated 10 percent of patients with acute ischemic stroke have a proximal large artery occlusion in the anterior circulation and present early enough to qualify for mechanical thrombectomy within 6 hours (Campbell et al. 2006; Furlan et al. 2015; Cohen et al. 2015; Chia et al. 2015), while approximately 9 percent of patients presenting in the 6 to 24 hour time window may qualify for mechanical thrombectomy (Jadhav et al. 2018). Second, only a few stroke centers have sufficient resources and expertise to deliver this therapy (Josephson et al. 2018). However, eligible

patients can receive standard treatment with intravenous tPA if they present to hospitals where thrombectomy is not an option, and those with qualifying anterior circulation strokes can then be transferred, a strategy called "drip and ship" (Sheth et al. 2015), to tertiary stroke centers where intra-arterial thrombectomy is available.

The antiplatelet medicines aspirin is effective for preventing ischemic stroke. It is used as first-line treatment after a noncardioembolic stroke, meaning a stroke in which the blood clot did not originate from the heart (Caplan et al. 2006). Aspirin has the advantage of being less expensive than the other antiplatelet medications. Possible side effects of aspirin include stomach upset and gastrointestinal bleeding. Also, one of the major drawback with aspirin treatment is its resistance i.e. inability of aspirin to reduce platelet activation and aggregation of thromboxane A2, 40% of aspirin users are resistant to aspirin, due to genetic polymorphisms of COX-1 and other genes involved in thromboxane biosynthesis (Hankey et al. 2006). Clopidogrel (brand name: Plavix) is another antiplatelet medication that can be used after stroke to reduce a person's risk of having another stroke. Compared with aspirin, clopidogrel causes a slightly higher frequency of rash and diarrhea, and a slightly lower frequency of stomach upset and gastrointestinal bleeding (Caplan et al. 2006; Hutton et al. 2005).

In some cases, aspirin and clopidogrel are used together for the first 90 days after an ischemic stroke. This strategy is called "dual antiplatelet therapy." After 90 days, however, the treatment is changed so that only one of these antiplatelet medications is continued. Long-term treatment using clopidogrel in combination with aspirin is not usually recommended after a stroke because the combination is no more effective for preventing another stroke than either clopidogrel or aspirin alone, while using the two in combination

increases the risk of bleeding in the brain. However, certain people who have had a recent heart attack or other acute coronary syndrome, or recent heart stent placement, are often treated with the combination of clopidogrel plus aspirin (Caplan et al. 2006; Caplan et al. 2013; Hutton et al. 2003; Hutton et al. 2005).

1.8 Possible treatment strategy for ischemic stroke

1.8.1 Piracetam

Clinical studies compared the piracetam and aspirin for the treatment of ischemic stroke, piracetam found to be safer but less efficacies than aspirin in the prevention of secondary stroke (Platt et al. 1993 and Grotemeyer et al. 2000).

Table 1.2: Piracetam versus acetylsalicylic acid in secondary stroke prophylaxis

Adverse events	Aspirin (N=307, 200 mg)	Piracetam (N=256, 1600 mg)
Gastrointestinal disorders (stomach upset, duodenal or gastric ulcer, gastritis and gastrointestinal bleeding)	13.8 %	7.4 %
Eczema and allergy	1.4 %	0.4 %
Heartburn	0.7 %	0.4 %

The LD-50 of piracetam is 5600 mg/kg (oral) in rats and safe clinically up to 24 g/day, while the LD-50 of aspirin is 200 mg/kg (oral) in rats and safe clinically up to 4 g/day (De et al. 1999). Piracetam is a nootropic, antiplatelet and a neuroprotectant which improves cerebral blood flow and oxygen availability in the brain by inhibiting thromboxane and stimulating

the production of prostacyclin (Nalbandian et al. 1983 and Winnicka et al. 2005). It has been reported that treatment with piracetam improved recovery from stroke (Yeo et al. 2017). However, there are no reports on brain penetration of piracetam during stroke condition which could be one of the critical factors influencing the pharmacological action of piracetam (Pillai et al. 2009, Savitz et al. 2007 and Latour et al. 2004). Therefore, we were interested to know whether the suboptimal efficacy of piracetam is due to its poor brain penetration or because of its mechanisms of action, as it is act through multiple pathways (Ahmed et al. 2010).

1.8.2 Biomaterial

A biomaterial is any substance that has been engineered to interact with biological systems for a medical purpose either a therapeutic (treat, augment, repair or replace a tissue function of the body) or a diagnostic one. Bioactive glass is a third generation biomaterial (regenerate & repair biological tissue) clinically used in bone and dental repair. Professor Larry Hench developed bioactive glass at the University of Florida in the late 1960s. The first bioactive glass used by Hench was 45S5 which is named as bioglass™.

Table 1.3: Bioglass™ or 45S5 composition

Elements	SiO_2	Na_2O	CaO	P_2O_5
Mole %	46.1	26.9	24.4	2.6

1.8.2.1 Application of bioactive glass based biomaterials

Bioactive glasses possess anti-inflammatory and antimicrobial application (Kargozar et al. 2019). In ischemic condition brain exposure of Bioglass significantly increased due to opening

of blood brain barrier (Pillai et al. 2009). Thus, availability of these anti-inflammatory agents in the brain reduces inflammation of neuron which will have significant value in the treatment of ischemia (Liu et al. 2014). Further, bio-resorbable glass fibers facilitate peripheral nerve regeneration (Bunting et al. 2005) and angiogenesis (Day et al. 2005). Thus, availability of bioactive glass in the brain facilitates regeneration of damaged nerve and formation of new blood vessel. Bioactive glass is safe as it has four FDA approval out of which three is for bone regeneration and one is for liver cancer treatment. In liver cancer treatment bioactive glass is used as a career for the loading of anticancer drug (Baino et al. 2018). In our current studies we have used barium and silver doped bioactive glasses. Barium blocks IRKs channels (Inwardly rectifying potassium channels) which regulate ADP-induced platelet aggregation (Alagem et al. 2001, Shankar et al. 2006). Silver inhibited ADP induced platelet aggregation by accumulating within platelet granules and reduces interplatelet proximity (Shrivastava et al. 2009). Thus, both barium and silver inhibits platelet aggregation and hold potential to be promoted as antiplatelet/antithrombotic agents. Further earlier studies reported that doping of metals like barium & silver in bioactive glasses significantly enhanced its VEGF stimulant property (Day et al. 2005).Therefore, in the present study we have prepared barium and silver doped bioactive glasses and evaluated them against ischemic stroke.

1.9 Origin of thesis

Piracetam has shown positive results in the preclinical studies but failed during phase III of clinical trial. This may be due to the issue of piacetam brain penetration. Thus we were intrested to know the pharmacokinetic and brain penetration of piracetam. Interestingly, we observed that the brain penetration was not a prime factor for its failure in the clinical study. At the same time, we were working on a collaborative project on bioactive glass as a bone implant. We observed that implantation of some of the bioactive glasses like barium and silver containing bioactive glasses have excessive bleeding compared to other bioactive glass implants. Then, we checked its anti-platelet property. We found anti-platelet property in barium and silver containing bioactive glasses, so this is serendipity. Therefore, we thought that this can be used for the prevention of secondary thrombosis, as clopidogrel. However, drug like clopidogrel also shows adverse gastro-intestinal events such as ulcer and bleeding. Therefore, we have performed anti-ulcer study. Hence, this was a serendipity and is more of an evidence-based study than conventional study. This was the first time we have shown this property of barium and silver containg bioactive glasses and further work is going on in our laboratory.

1.10 Hypothesis

The study focuses on application of bioactive glasses against both peripheral as well as brain ischemic reperfusion injury. Brain ischemic reperfusion injury was induced by middle cerebral artery occlusion while the peripheral ischemic reperfusion injury was induced by celiac artery occlusion. MCAO induces the platelet aggregation, reduces cerebral blood flow, EEG potential and sensory motor activity of the rats. Since, both barium and silver are reported to have antiplatelet property, silver inhibited ADP induced platelet aggregation by

accumulating within platelet granules and reduces interplatelet proximity (Shrivastava et al. 2009) while barium block IRKs channels (Inwardly rectifying potassium channels) which regulate ADP-induced platelet aggregation (Alagem et al. 2001, Shankar et al. 2006). Thus, both silver and barium inhibits platelet aggregation and hold potential to be promoted as antiplatelet/antithrombotic agents. Additionally bioactive glass is reported to have VEGF stimulation property thus in the present study we have hypothesized the barium and silver doped bioactive glass for the treatment of cerebral stroke. Further, cerebral stroke patient is more prone to ischemic ulcer and treatment with antiplatelet drug causes worsening of the gastroduodenal ulcer. Bioactive glass is reported to have antiulcer activity. Doping of barium in bioactive glass enhanced its bioactivity and thus may have greater anti ulcer property. Further, the acute and sub-acute toxicity studies of BaBG and AgBG suggested that BaBG is safe as compared to AgBG. Therefore, in the present study we have evaluate the potential of BaBG against ischemic and other ulcer models.

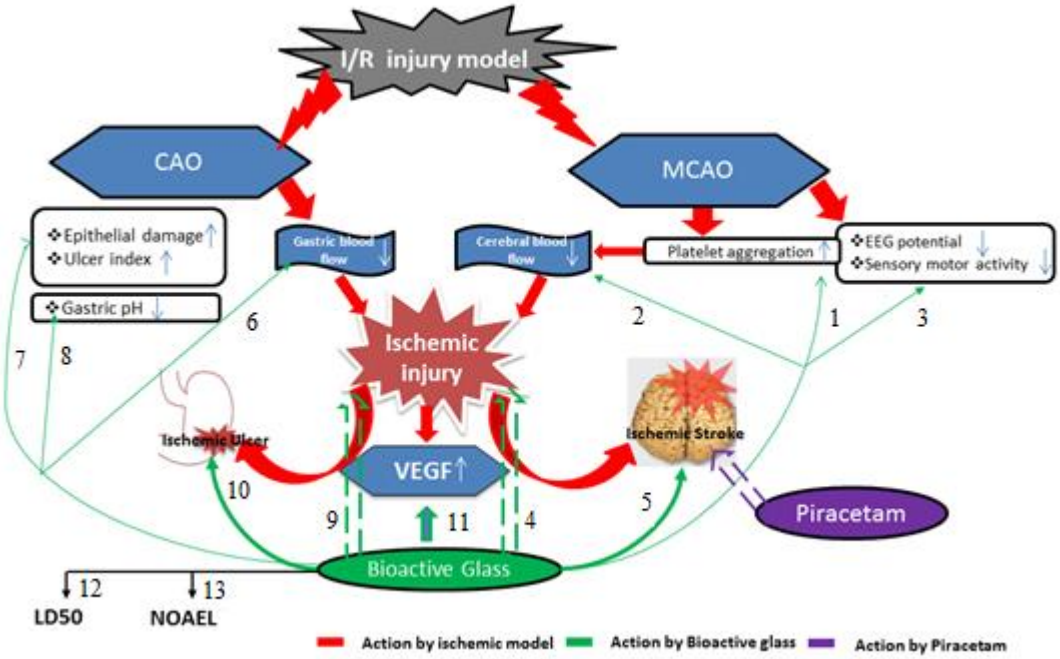

Figure 1.1 Proposed hypothesis; *The proposed hypothesis is based on the application of bioactive glasses against both brain as well as peripheral ischemic reperfusion injury. Brain ischemic reperfusion injury was induced by middle cerebral artery occlusion while the peripheral ischemic reperfusion injury was induced by celiac artery occlusion. MCAO induces the platelet aggregation, reduces cerebral blood flow, EEG potential and sensory-motor activity of the rats. Since both barium and silver are reported to have antiplatelet property, silver inhibited ADP induced platelet aggregation by accumulating within platelet granules and reduces interplatelet proximity (Shrivastava et al. 2009) while barium block IRKs channels (Inwardly rectifying potassium channels) which regulate ADP-induced platelet aggregation (Alagem et al. 2001, Shankar et al. 2006). Thus, both silver and barium inhibits platelet aggregation and hold potential to be promoted as antiplatelet/antithrombotic agents. Thus, we hypothesized that barium and silver containing bioactive glass will inhibits platelet aggregation (01) by recovering cerebral blood flow (02), EEG potential, sensory motor activity (03) and treatment with bioactive glass recovers from ischemic stroke injury (04 and 05) in the rats. Further, cerebral stroke patient is more prone to ischemic ulcer and treatment with antiplatelet drug causes worsening of the gastroduodenal ulcer. Bioactive glass is reported to have antiulcer activity. Doping of metal like barium in bioactive glass enhanced its bioactivity and thus may have greater anti-ulcer property. Thus we have hypothesized anti-ulcer potential of bioactive glass against CAO induced alteration in gastric blood flow (06), epithelial damage (07), gastric pH (08) and gastric ischemic injury (09) and ischemic ulcer (10) in the rats. Additionally bioactive glass is reported to have VEGF stimulation property and doping of metals like barium and silver in bioactive glasses significantly enhanced its VEGF stimulant property (Day et al. 2005). VEGF is associated with angiogenesis and vascular remodeling*

(Gandin et al. 2016). Increase in VEGF level has been reported to aid in recovery of pathological as well as both neurological deficits in MCAO rodent model (Gandin et al. 2016). Therefore in the present study we have hypothesized barium and silver doped bioactive glass will increase VEGF level in the brain and stomach ischemic region (11) and evaluated them against both brain as well as peripheral ischemic reperfusion injury rodent model. Further, the acute and sub-acute toxicity studies of BaBG and AgBG evaluated to determine their safety (12 and 13).

1.11 Objectives of the thesis

Objective 1. Pharmacokinetic and brain penetration study of piracetam in focal cerebral ischemic rats.

Objective 2. Pharmacological effect of barium containing bioactive glass (BaBG) for the treatment of cerebral ischemic reperfusion injury.

Objective 3. Pharmacological effect of silver containing bioactive glass (AgBG) for the treatment of cerebral ischemic reperfusion injury.

Objective 4. Acute and subacute toxicity studies of BaBG & AgBG in Wistar rats

Objective 5. Pharmacological effect of barium containing bioactive glass in ischemic and other gastro-duodenal ulcers