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POTENTIAL HERBAL DRUGS FOR ISCHEMIC STROKE : A REVIEW

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Abstract

Cerebral stroke is the second most leading cause of mortality and morbidity accompanied by loss of blood supply to the particular area of brain. Increased level of reactive oxygen species and cell debris is driving factor for stimulating multiple signaling mechanisms. The multifarious pathophysiology of this disabling disease, multifactorial effects of active constituents of herbal medicine, along with, lack and demerits of clinical treatments may advocate the favorable inclination to natural drugs for treatment. Herbal based treatments are efficacious and assumed to be most effective for chronic health conditions like stroke. The tremendous properties of herbal drugs such as anti-apoptotic, anti-inflammatory, anti-oxidative might be an ultimate therapeutic approach for the cerebral stroke. Active constituents of herbal drugs showed the promising results in the pre-clinical studies but researchers have been unable to transform these results from animals to human use creating a huge challenge and questioning the efficacy of the herbal drugs and their use in the cerebral stroke. Attention should be paid to herbal drugs which have extensive pharmaceutical window and appropriate pharmacological targets with few adverse effects. Moreover, lot of attention should be given to transform the success of animal research to human use. The present review focused on different medicinal plants and drugs that have been tested in animal models of stroke.

Keywords : Stroke; Pathophysiology; Anti-oxidative; Herbal drugs; Anti-apoptotic; Anti-inflammatory.

Introduction

Cerebral stroke is the second most leading cause of mortality and affects upto 8, 00,000 US people yearly. The health care expenses on the stroke are $ 34 billion annually which results as loss of productivity and disability. From the decades of research, t-PA is the only drug used by researchers for the treatment of stroke, but it has limited pharmaceutical window and associated with harmful adverse effects like hemorrhage. Only a few numbers of patients or individuals are taken t-PA (tissue plasminogen activator or Alteplase) due to its ineffective therapeutic properties (Reed *et al*., 2001). tPA is effective only in the opening up of occluded cerebral vessel and consequently has useful outcomes after ischemic stroke. Despite of the various researches, no single drug is found to be effective in attenuating the cascade of pathogenic process in ischemic stroke. Ischemia leads to accumulation of reactive oxygen species and cell debris which is the driving forces for the initiation of several signaling pathways such as oxidative, inflammatory and apoptotic and activation of microglias, astrocytes leads to the progression of the pathogenesis of disease (Moskowitz *et al*., 2010). 85 % of stroke is ischemic and rest 15% is hemorrhagic, therefore the use of neuroprotective agents in both types with the attenuation of pathogenic cascade mechanisms will be the useful strategy. In hemorrhagic stroke, other factors such as physical damage and excitotoxicity can occur (Zemke *et al*., 2007). Due to the lack and adverse effects of the allopathic treatments, researchers now focus on the herbal based drugs with anti-inflammatory, anti-apoptotic, and anti-oxidative properties for the treatment of stroke. The tremendous properties of active constituents of herbal drugs are proven useful in the animal studies but have limited use in human. Therefore, understanding the efficacy of these drugs and transformed the results from animals to human use must be challenging (Ginsberg *et al*., 2009). The review focuses on the distinctive herbal drugs which are having prominent role in the animal models of stroke.

Pathophysiology of ischemic stroke

Hypoxia is the disruption of oxygen supply to the tissue whereas ischemia is the loss of the blood supply (oxygen and nutrients) to the particular area of the brain. Necrosis of the core infarcted tissue, maintenance of metabolism and reduce the functions of penumbra tissues are the initial events to be occurred after ischemic insult (Broughton *et al*., 2009). Due to the lack of supply of ATP and Na^+/K^+ transporter results in the excessive influx of the Ca^{2+} leads to the initiation of apoptotic pathway and cell death. Excitotoxicity due to influx of calcium is the result of the accumulation and activation of glutamate NMDA and AMPA receptors. Increased production of oxygen radicals (reactive oxygen species) also facilitates the initiation and progression of pathogenic signaling pathways includes oxidative, and inflammation due to lacking of scavenger mechanism in the ischemic condition (Love, 1999). Abundantly present astrocytes and microglias are also activates as a result of cell debris leads to upregulation of the arachidonic acid metabolism, increased expression of inducible nitrate synthase results to the

accumulation of oxygen radicals in acute stroke. Inflammation plays a prominent role in the ischemic insult. Release or secretion of various cytokines, chemokines along with the infiltration of leucocyte, neutrophils is a result of activated microglias and astrocytes. Activated type 1 microglia is found to releases and increases the expression of pro-inflammatory mediators along with the stimulation of signaling cascade that contributes towards the transcription of pro-inflammatory genes (Rothwell *et al*., 2003). Edema in the cerebellum after the ischemic stroke also plays a dominant role. Dysfunctioning of various cations, transporters i.e. sodium hydrogen anti-porter family, sulfonyl urea receptor-1 transient receptor potential melastatin 4 (SUR1-TRPM4) and aquaporin- 4 is crucial in amplifying the cerebral edema after stroke (Stokum *et al*., 2016).

Targets for neuroprotection in stroke

Inflammation

Numerous studies have conducted on the neuroprotection in ischemic insult via anti-inflammatory mechanisms. Inflammation plays a dominant role in ischemic condition. After ischemia, accumulation of radicals and cell debris amplifying the release of pro-inflammatory cytokines, chemokines and inflammatory pathways leads to the progression of the pathogenesis of disease (Lakhan *et al*., 2009; Jim *et al*., 2010). Inflammatory process also includes the activation of the matrix metalloproteinase (MMP) that is disrupting the BBB and cause edema due to the dysfunctioning of various transporters and cations. It has been studied that inflammation provides both good and bad effects in the ischemic condition and this statement is the topic for debate. The bad effects of inflammation superimpose the good effects at the early stages of the stroke. An important inflammatory mediator called tumor necrosis factor alpha (TNF-α) interacts with their R1 and R2 receptor leads to the mediation of death signals with the interaction of Fas associated death domain (FADD) and progresses the inflammation via NFkB genes (Jablonska *et al*., 2011). The NFkB pathway or signaling mechanism results to the transcription of several inflammatory mediators out of which interleukins found to play a prominent role. IL-1 mediates inflammation with the binding to the IL-1 receptor and results in the excessive release of interleukins that worsens the ischemic condition. Antagonist of IL-1 receptor or inhibitors of caspase activation might be the effective therapeutic approach for neuroprotection. Another target for neuroprotection will be Tnf-α and several signaling mechanisms which may be useful strategy for attenuating the ischemic inflammation (Pradilo *et al*., 2012). Microglias found to be neuroprotective which scavenging the toxic molecules and the drugs which activates the microglias might be provide neuroprotection. The process of inflammation in stroke not only damage to the brain it also stimulates various signaling mechanisms that contributes to the further damage.

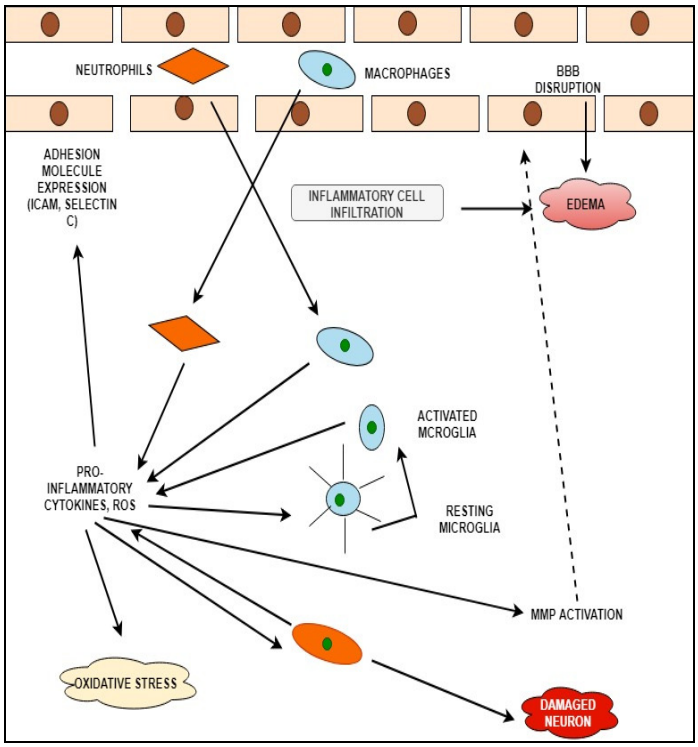

Fig. 1: Downstream pathway of activated pro-inflammatory cytokines and inflammatory mediators lead to ischemic stroke.

Oxidative stress

Production of the free radicals and reactive species is a result of not only inflammation but also excitotoxicity (Pradeep *et al*., 2012). Hydroxyl radical, peroxynitrile molecules are very reactive and cause damage to the lipid cell membrane leading to cell death. Neutralizing the production of free radicals is the way of reducing oxidative stress. Nitric oxide has beneficial effects in stroke initially and it is normal signalling molecule. But increased activity of iNOS as discussed earlier lead to excessive release of NO and leads to the aberration in the signaling mechanisms. Excessive NO interacts with peroxynitrile and cause damage. Study have conducted on Nebivolol in CCAO rats and results shown that after subjection of Nebivolol it reduces the expression of iNOS and amplifies the expression of beneficial eNOS (endothelial nitrate oxide synthase) (Heeba *et al*., 2012). Another source of ROS is NADPH oxidases and the specific inhibitors of this enzyme must be used as treatment of stroke (Radermachar *et al*., 2013). Various studies have conducted in MCAO rats in which oxidative stress is the major target. HS gas is found to increase the activity of antioxidants such as superoxide dismutase and glutathione peroxidase in ischemic rats. Inhibition of downstream signaling mechanisms leads to oxidative stress might be beneficial after ischemic condition. Study on antioxidant N-tertbutyl-α-phenyl nitrone in mice has conducted and a result reflected that it suppresses the component 3 which mediates the oxidative stress and expression of oxidative signaling mechanisms (Yang *et al*., 2013).

Blood brain barrier (BBB) disruption

Matrix metalloproteinases (MMP) – 2 and MMP – 9 are associated with the disruption of BBB after the cerebral ischemic insult. MMP – 2 activate and cleave the MMP -9. It also stimulates the activity of MMP – 9 which can disruption, loosening of the vascular wall a thus damage BBB. Tissue

inhibitor of matrix metalloproteinase (TIMP) mediates the activity of metalloproteinases endogenously and the drugs that activate the TIMP might be a therapeutic approach in stroke. Various studies have conducted to reduce the expression and activity of MMP-2 and MMP-9. Hyperbaric oxygen treatment improved the embolism in rat model after modulation of the expression of MMP-9 but it is not effective when given with tPA (Michalski *et al*., 2012). Treatment with ethanol also decreases the activity of MMP-2 and MMP – 9 in MCAO rats. It also reduce the cerebral edema by modulating the activity of MMP-2 associated various transporters (Zeng *et al*., 2012). Study on Doxycycline and Kruppel-like factor 2 (KLF2) stimulates the expression of tight junction proteins in MCAO rats (Shi *et al*., 2013). SP600125 which is an inhibitor JNK restores the tightening of the vascular junction and reduced the BBB damage (Chen *et al*., 2012).

Excitotoxicity

During ischemic insult, toxic amount of neurotransmitters releases into extracellular space due to the depletion of neuronal oxygen and energy stores. Activation of glutamate receptor such as AMPA and NMDA results in the excessive release of calcium leads to the activation of several calcium mediated signaling cascade which initiates the process of necroptosis and autophagy. Excitotoxicity mediated by the glutamate plays a dominant role in the pathogenesis of cerebral ischemia. Reduce or block the activity of glutamate or its receptors might be a neuroprotective approach in ischemia. Researchers have conducted a study on extract of Ginkgo biloba on MCAO rats and concluded that Ginkgo biloba reduced neurodegeneration and edema by declined the levels of extracellular present glutamate (Mdzinarishvili *et al*., 2012). Another approach for the neuroprotection is blocking the action of glutamate receptors by silencing techniques. The micro RNA mir-223 reduces the expression of different subunits of glutamate receptor 2 and NR2B and found to be provide neuroprotection in global ischemia (Harraz *et al*., 2012). Stimulation of the transient receptor potential vanilloid 4 (TRPV4) increases the expression and activity of NMDA receptor. Researcher uses the antagonist of TRPV4 in tMCAO mice and suggested that it reduces the infarct size. Clinical studies and trials such as i.v. magnesium efficacy in stroke (IMAGES) and filed administration of stroke therapy (FAST-MAG) have conducted and these trials are under processing and might be a useful therapeutic approach in ischemia (Saver *et al*., 2014).

Apoptosis

Loss of blood supply in the ischemic condition leads to the disruption of oxygen and glucose to the brain. The depletion of energy stores along with reduced metabolism initiates many factors which are discussed above out of which apoptosis and necrosis plays a prominent role. Cell death either by apoptosis or necrosis initiates by the depletion of energy and metabolism. We can say that apoptosis is beneficial to necrosis because it can be prevented by giving therapeutic approaches which blocks the apoptotic pathways at multiple steps. The core cells died of necrosis while the penumbra cells are died of apoptosis (Majid, 2014). ATP is the driven force for the initiation of apoptosis, means cells with sufficient ATP can die of apoptotic mechanisms and those with insufficient ATP are the subjects of necrotic death. The mitochondrial pathway of apoptosis is shown in figure 2. The pathway can be distinguished into caspase dependent or caspase independent. Release of cytochrome C followed by the caspase C activation in caspase dependent signaling leading to the generation and initiation of caspase mechanisms. Strategized the neuroprotective therapies with caspase 3 blockade activity might be an effective therapeutic approach. Researchers have conducted a study on drug molecules such as Trashninone II A, Diallyl sulfide which has the property of inhibiting cleaved caspase and reduced expression of caspase 3 respectively in tMCAO rats and concluded that these molecules inhibiting apoptosis with caspase 3 blockade along with increasing the levels of anti-apoptotic protein i.e. BCL-2 (Zgacv *et al*., 2013). Other pathways are also contributing to the cell death but the mitochondrial pathway which is accompanied by the release of apoptosis inducing factor (AIF) and activated by poly ADP ribose polymerase (PARP) is dominant and neuroprotective approaches that alters mitochondrial pathways are investigated (Fu *et al*., 2015).

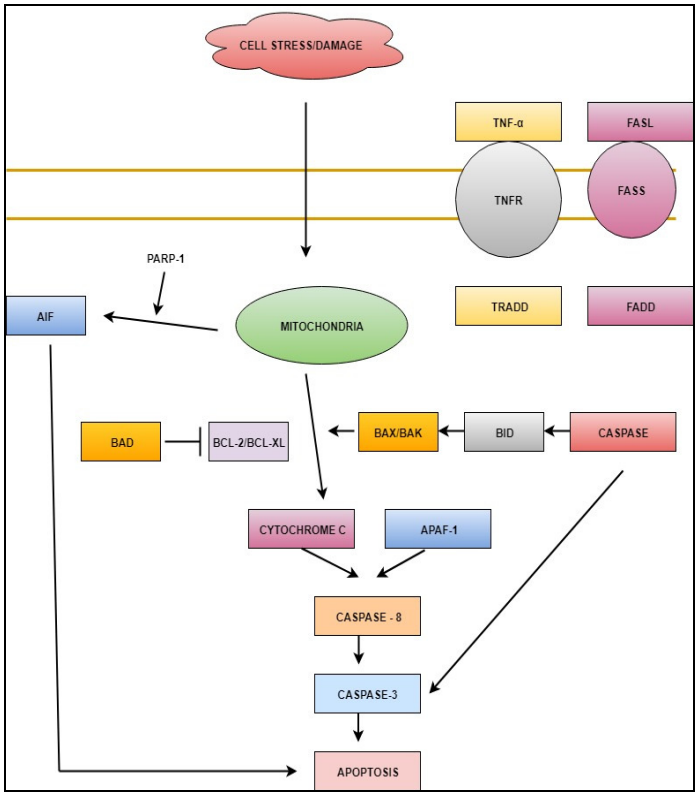

Fig. 2: Activation of caspase dependent apoptotic pathway followed by the release of mitochondrial cytochrome C and signaling of death receptors.

Autophagy

Mechanism of the autophagy in the ischemic stroke is not fully understood. It serves as dual role in ischemic condition. It provides protective mechanism by absorbing deleterious components in some cells and on the other hand itself harmful to another cell. The autophagy response is mediated by the sensing pathways, ATG protein family and the drug rapamycin (Zenke *et al*., 2007; Gabryel *et al*., 2012). Programmed cell death i.e. apoptosis in ischemia is inhibited by the autophagy which is considered to be beneficial. Autophagy is also induced by inhibiting the mTOR pathway with rapamycin. Researchers has conducted the study and concluded that rapamycin inhibits apoptosis and cell death in sub-arachnoid hemorrhage of rat model. It was also found that inhibition of autophagy facilitates the brain damage (Jing

et al., 2012). Another approach for the induction of autophagy is increases the activity of tuberous sclerosis complex (TSC) which leads to the mTOR inhibition. It has seen that with the suppression of TSC1 subunit brain is vulnerable to cell death after ischemic insult in vivo. In a nutshell, autophagy is neuroprotective in ischemia (Papadakis *et al*., 2013).

Neuroprotective stratigies for ischemic stroke

Natural compounds: A Promising Neuroprotectants

Currently, treatments are inadequate for clinical use, tPA being globally endorsed for treating ischemic stroke has limited time window, and therefore it is restricted to only few patients (Lo 2010; Moskowitz *et al*., 2010). Therefore an effective treatment has to be discovered with extended time window as an alternative to tPA. Reperfusion that may seem to be protective accompanies free radicals and cytokines-mediated inflammation. An effective drug therapy may help to deal with stroke. Herbal medicines have shown prominent effect on treating various inflammatory diseases. Chinese herbal medicine and other natural products are being used from thousand years in clinical treatment to fight inflammation and protect neurons stricken with ischemic stroke. These many natural compounds are still used as healthy food supplements. There are 55 herbal compounds that may be effective against ischemic stroke as they bear anti-inflammatory and anti-oxidant properties. These active compounds are classified into phenolic compounds, saponins, terpenoids and alkaloids based upon their chemical structures, sources and their pharmacological action.

Phenolic compounds

Phenolic compounds are eminent amongst large group of herbal medicines. Their capability of entrapping singlet oxygen makes them anti-oxidant, anti-inflammatory as well as neuroprotective against cerebral ischemia-reperfusion injury. Flavanoids are the distinct phenolic compounds found in many vegetables and medicinal herbs. Based on their chemical structures they have been divided into several groups such as: isoflavones, flavonols, flavononones and flavones. Flavanoids and other phenolic compounds mechanism of action is mainly antioxidant and anti-inflammatory associated with free radical neutralization and inhibition of inflammatory factors. The pharmacological actions can be potentiated by modifying functional group by hydroxylation or methoxylation. Puerarin is an augmented isoflavone derived from Pueriae Radix, has found to be neuroprotective in a rat brain stricken with ischemic injury and its mechanism for neuroprotection is associated with erythropoietin (EPO) elevated expression and decreased COX-2 level (Gao *et al*., 2009; Lim *et al*., 2013). Genistein is an isoflavone derived from soyabean had found neuroprotective against I/R injury as it hampered the NADPH oxidase activity (Ma *et al*., 2010; Castello-Ruiz *et al*., 2011). Nieman *et al*. reviewed that Flavonols belonging to another group of flavonoids is an apprehensive treatment for stroke, diabeties and heart diseases. (Nieman *et al*., 2012). A commonly found flavanol known as Quercetin is found in green vegetables, onions, broccoli and medicinal herbs bearing broad bioactivities. Ghosh *et al*. evaluated the effect of nano encapsulated Quercetin on ischemia-reperfused brain and the results showed decreased expression of MMP-9 and iNOS that ultimately reduced the hippocampal damage in young as well as aged rats (Ghosh *et al*., 2013). Gelderblom *et al*. well evaluated another flavonal compound Fisetin found abundantly in parrot tree remarkably provided protection against brain injury due to cerebral by lowering the production, release as well as infiltration of various inflammatory factors such as TNF-α, dendritic cells and macrophages (Gelderblom *et al*., 2012). Baicalin, another neuroprotective and anti-inflammatory flavanoid compound that promoted differentiation of neural stem/progenitor cells (Li *et al*., 2012). Zhang *et al*. from his experimental study accounted that the protection aided by luteolin from cerebral I/R injury of stroke animal model was the result of Nrf-2 activation that transcribes HO-1 (Zhang *et al*., 2013). Lee *et al*. also dealt with one of the phenolic compound name cinnamophilin to study its protective effect on stroke model. Cinnamophilin inhibited the production of COX-1 and 5-lipoxygenase during ischemia and reperfusion thereby rendering protection (Lee *et al*., 2009). Another active ingredient of medicinal herb St John's wort is Hyperforin that was being investigated for neuroprotective effect by Albert *et al*. on a transient focal cerebral ischemia of rat model. He found that hyperforin provided protection through COX-1 and 5-lipoxygenas inhibition. Lin *et al*. further studied the hyperforin mechanism of protection and reported that the protection by hyperforin was due to increased activity of transient receptor potential cation channel, phosphorylated cAMP response element binding protein (p-CREB), subfamily C, member 6 (TRPC6) (Lin *et al*., 2013).

Saponins

Saponins are relatively big molecules having detergent property. Normally they can't easily pass BBB, however disruption of BBB during cerebral ischemia and reperfusion makes saponin enter into parenchyma. Saponins found in many medicinal herbs are well-known for its strong anti-inflammatory property against multiple infectious and autoimmune diseases. Gigenosides are best-studied saponins from plant Panax ginseng involving ginsenoside Rb1, Rg1, Rg3, Rd and etc. Studies have revealed its neuroprotective role in stroke. For example Ginsenoside Rb1 given intravenously to permanent cerebral ischemic rats resulted in 50 % infarction size reduction as compared to vehicle control (Zhang *et al*., 2005). Yuan *et al*. also evaluated and reported that ginsenoside Rb1 induced protection in brain of ischemic model via stimulation of GDNF expression (Yuan *et al*., 2007). Tain *et al*. also assessed and revealed that Ginsenoside Rg3 action on rat induced with cerebral I/R appreciably reduced infarction and also improved neurological shortfall (Tian *et al*., 2005; Shin *et al*., 2013). The mechanistic behind ginsenoside Rg3 protection was inhibition of Ca^{2+} and H_2O_2-induced swelling of mitochondria, ROS generation and enhanced mitochondrial activity and ATP levels (Tian *et al*., 2009). In focal brain injury induced by I/R, the protective role of Ginsenoside Rd was evaluated and the outcome was reduced iNOS and COX-2 level (Liu *et al*., 2009; Ye *et al*., 2011). Ye *et al*. reported that Ginsenoside Rd has been verified for its neuroprotective role in both animal and humans. It displayed appreciable effect on aged rat of ischemic stroke model by reducing infarction volume and improving neurological disability when administered after reperfusion (Ye *et al*., 2011). Liu *et al*. had reported the clinical efficacy of ginsenoside Rd in patients of acute ischemic stroke as examined in a randomized and placebo-controlled phase II clinical trial. The results obtained from ginsenoside Rd treated group found statistically significant as

compared to the placebo group (Liu *et al*., 2009). On the other hand ginsenoside Rd brought no improvement in neurological functioning; therefore it was not possible to develop it as clinical drug. Guan *et al.* reported that ruscogenin; a component of Chinese herb Ophiopogon japonicas was tested for its neuroprotective and anti-inflammatory properties in focal brain of I/R mouse model. The mechanism behind protection of Ruscogenin was the inhibition of NF-κB p65 expression and its cytosolic translocation to nucleus; this ultimately had remarkable effect on infarction size and neurological deficits (Guan *et al*., 2013). A component of Astragalus membranaceous known as Astragaliside IV has found to be effective in reducing infarct volume when administrated at the start of reperfusion (Li *et al*., 2012). The mechanism of neuroprotection was linked with the NF-κB suppression and also the suppression of its all inflammatory factors such as MMP-9, ICAM-1, and TNF-α in the parenchyma of brain (Li *et al*., 2012, 2013).

Terpenoids

Terpenoids, five-carbon isoprene units is the largest group amongst natural products. Numerous terpenoids possesses stronger pharmacological activity as anti-oxidant, anti-inflammatory as well as neuroprotective. Resveratrol is one of enriched terpenoid component found in fruits and vegetables and especially in red wine showed neuroprotective action on ischemic brain by decreasing the level of MMP-9 and NO in ischemic brain and apoptotic cells (Gao *et al*., 2006; Tsai *et al*., 2007; Li *et al*., 2012). Ha *et al*. tested the role of ginger terpenoid known as 6-shogaol on transient global cerebral ischemia model and the results revealed that 6-shagol notably decreased the LPS- induced increased iNOS expression along with increased PGE-2 IL-1β, COX-2 and TNF-α in microglial cells (Ha *et al*., 2012). Another terpenoid compound tetrahydroxystilbene obtained from Chinese herb "Polygonum multiflorum" had prominent effect on rats brain induced with 90 min ischemia and reperfusion of 24 hr. Tetrahydroxystilbene appreciably reduced the infarction lesion and neurons loss. In order to study the mechanism behind protection Wang *et al*. evaluated test on rat cultured cortical neurons that was exposed with OGD and reoxygenated to mimic ischemia-reperfusion and he found that protection was due to inhibition of iNOS mRNA expression and NF-κB inhibition (Wang *et al*., 2009).

Alkaloids

Alkaloids are found in nearly all the herbal plant have major bioactivities. Alkaloidal compounds such as huperzine A, sinomenine, ligustrazine have shown nueroprotective as well as anti-inflammatory action in several preclinical studies. Wang *et al.* assessed the nueroprotective activity of Huperzine A derived from Huperzia serrata herb against cerebral ischemia of 2 hr followed up by 24 hr reperfusion in rats. Huperzine A reported to reduce brain infarction and neurological shortfall. The protection by Huperzine A was due to inhibition of NF-κB translocation to nucleus and reduced iNOS and COX-2 expression in cortex and striatum (Wang *et al*., 2008). Another study conducted by Loh *et al*. found that compound Leonurin from plant Leonotis leonurus provided protection from I/R induced cerebral infarction and neurological deflict by appreciably reducing level of ROS in mitochondria thereby restoring mitochondria's membrane potential (Loh *et al*., 2010; Qi *et al*., 2010). Similarly Wu *et al*. assessed Sinomenine obtained from plant Sinomenium acutum neuroprotective action on rat model of cerebral ischemia-reperfusion. Sinomenine showed prominent effect on infarction size increased due to ischemia and recovered the neurological function (Wu *et al*., 2011). Another study accomplished by Kao *et al*. proved that alkaloid ligustrazine exhibited neuroprotective effect on cerebral ischemia. The protection was interceded by Ligustrazine up regulation of Nrf-2 that resulted in inhibition of macrophages and infiltration of immune cells (Kao *et al*. 2013).

Other approaches: Neuroprotective treatments with Pleiotropic effects

Carnosine

Carnosine is a natural dipeptide compound possessing both antioxidant and anti-excitotoxic properties (Boldyrev *et al*., 2010). It's also a chelating agent of metals like zinc necessary for matrix metalloproteinase activity. Because of its ability to cross readily the blood-brain barrier makes it prominent therapy for early stages of stroke. Bae *et al*. has revealed in his study that carnosine brings neuroprotection in both transient as well as permanent ischemic animal model (Bae *et al*., 2013). Krishnamurthy *et al*. found Carnosine as anti-exitototoxic in pMCAO mice that helped to reduce infarction size and improved neurologic function. It also altered the MMP activity along with the levels of reactive oxygen species. Carnosinase is an enzyme that cleaves Carnosine into alanine and histidine aminoacids that showed neuroprotection in pMCAO mice model which was later inhibited by Bestatin, an inhibitor of carnosinase (Krishnamurthy *et al*., 2009).

Asiatic Acid

Asiatic acid found to be beneficial in wound healing, liver injury and toxicity due to beta-amyloid, as it worked on oxidative stress, inflammation, and excitotoxicity. It is also found to be neuroptotective in stroke as recommended in recent studies. In pMCAO model of mice, Asiatic acid reduced the infarction size and helped to improve neurologic insufficiency, probably by suppressing mitochondrial damage and protecting BBB. Lee *et al*. also found Asiatic acid to be neuroprotective in ischemia rat model as it inhibited mitochondrial damage and MMP-9 activation (Lee *et al*., 2012). An extract from Centella asiatica containing acetic acid has shown neuroprotective effect in tMCAO rats. Centella asiatica showed antioxidant by and improved behavioral function (Tabassum *et al*., 2013).

Flavonoids: Xanthohumol

Xanthohumol is a flavanoid component with broad therapeutic activity such as anti-inflammatory, anti-apoptotic, antioxidant, and antithrombotic. Xanthohumol antioxidant effect was evaluated in a study conducted on MCAO induced ischemia in rats that revealed its ability to reduce the iNOS expression and all the levels of inflammatory factors such as TNF α, hypoxia inducible factor 1 alpha (HIF-1 α). Also the expression of activated caspase 3 and hydroxyl radicals was reduced in xanthohumol treated rats. Another flavanoid constituent Nargingenin aided neuroprotection by giving antioxidant and anti-inflammatory effect in tMCAO rat (Raza *et al*., 2013). In tMCAO mice, the protection favored by Fisetin, a flavanoid component against brain ischemic injury was due to inactivation of inflammatory cells and inhibition

of macrophages and dendritic cells migration into the brain (Gelderblom *et al*., 2012).

Cannabinoids

Cannabinoids is one of the major compounds having multiple beneficial effects especially anti-inflammatory effects in stroke (Zhou *et al*., 2012). However, evidence also suggests Cannabinoids possessing antioxidant and antiapoptotic properties Cannabinoids receptor agonist, WIN55, 212-2 and JWH-133 had shown significant reduction in the size of brain infarction and neurological mutilation, as well as oligodendrocyte precursor cells protection due to activation of microglia and macrophages in ischemic mice and rats. Sun *et al*. also studied the effect of TAK-937 another cannabinoid receptor agonist that provided protection of brain in tMCAO rats when given at the time of hypothermia by reducing the volume of infarction (Sun *et al*., 2013).

The therapeutic advancement of herbal drugs by a lot of scientific enterprise has proved its significance in treating cerebral ischemic conditions. Here are some of the compounds with detailed description, mentioned in the following section that may have significant therapeutic benefit.

Resveratrol

Resveratrol, a phytoalexin (3, 4, 5-trihydroxystilbene) is found mainly in skin and grapes seed and is reported to have broad pharmacological activity on inflammation, oxidative stress and carcinogens. The antioxidant property of is revealed in the study of ischemic-reperfusion stroke model pretreated with resveratrol (Sakata *et al*., 2010). Another study by Ren and his co-workers who used MCAO rat model to determine the neuroprotective effect of resveratrol and the result suggested that resveratrol provided protection via upregulation of Nrf2/AR. (Ren *et al*., 2011). Yosuf *et al*. also studied the effect of resveratrol in MCAO model against mitochondrial dysfunctions in the rat's hippocampus. The study revealed that resveratrol apparently helped in restoration of ATP and reduced release of cytochrome c from mitochondria that resulted marked decrease in DNA fragmentation. Brain infarction and edema was also reduced (Yosuf *et al*., 2009). Further, Simao *et al*. investigated the possible relation between resveratrol nueroprotective effect and pathway of the apoptosis/ cell survival in rat model of global cerebral ischemia pursued by discrete periods of reperfusion. Resveratrol pretreatment rendered protection by increasing Akt, GSK-3β and CREB phosphorylation in the CA1 hippocampus that are responsible for regulating various cellular processes, such as cell proliferation, glucose metabolism, apoptosis, transcription. The protection was reversed by PI3-K inhibitor, LY294002 (Simao *et al*., 2012).

Ginkgo biloba

The medicinal property of Ginkgo biloba extracts has been reported for their health benefits, specifically in the brain. The antioxidant properties of Ginkgo extract, EGb761, is protective for neurons against oxidative stress as revealed in the study EGb761 treatment resulted in an increased activity of catalase and SOD and decreased LPO activity in the hippocampus, striatum and substantia nigra of rats brain (Birdi *et al*., 2001). Tulsulkar *et al*. studied the result of EGb761 in mice model of transient global ischemia and found that EGb761 pretreatment decreased hippocampal neuronal death induced by ischemia and inflammation; it also reduced DNA fragmentation of hippocampal neurons. The protection was mediated due to increased expression of Nrf2, HO1, and vascular endothelial growth factor (VEGF) suggesting that neuroprotection by EGb761 was HO1/Nrf2 pathway mediated (Tulsulkar *et al*., 2013). Another neuroprotective effect of EGb761 was observed in mice subjected with permanent distal middle cerebral artery occlusion (pMCAO). In EGb761 treated mice, the cerebral infarction and neurologic deficit score (NDS) was lowered due to increased expression of HO1, VEGF and eNOS in the brain cortices (Shah *et al*., 2011).

Activity of Na/K-ATPase is being concerned with the cerebral ischemic-pathophysiology. EGb761 showed neuroprotective effect in ipsilateral cortex of mice subjected to middle cerebral unilateral occlusion by increasing ATPase activity as compared to the non ischemic contra lateral cortices group [51]. EGb761 also managed to reduce the cellular edema and neurodegeneration.

Another study revealed that the mechanism behind the nueroprotection aided by EGb761 in MCAO induced ischemia in mice brains is due to reduction of excitotoxicity that ultimately reduced the cell swelling in hippocampal slices and cell degeneration. Further Mdzinarishvilli *et al*. evaluated the relation between calcium and cerebral ischemic injury by EGb761 administration in rats during ischemia and the result indicated that EGb761 reduced the intracellular calcium that caused excitotoxicity by decreasing glutamate and aspartate concentration and increasing GABA concentration (Mdzinarishvilli *et al*., 2012). Yang *et al*. demonstrated the effect of an active component named Ginkgolide B, a terpene lactone extracted from Ginkgo biloba leaves in MCAO rats and it revealed that Ginkgolide B reduced cerebral infarction volume by decreasing the concentrations of glutamate, aspartic acid and glycine and increasing the extracellular GABA concentrations in rats brain (Yang *et al*., 2011). Another active component of Ginkgo biloba is Ginkgolide K which is a natural platelet-activating factor receptor antagonist. Ma *et al***.** investigated its neuroprotective role in cerebral ischemia of MCAO model and found that ginkgolide K reduced the volume of infarction by strikingly reversing the level of MDA, NO, NOS and SOD to their normal state in ischemic brain as well as in serum (Ma *et al*., 2012).

Curcumin

Curcumin, as one of the popular Indian spice known as turmeric belongs to the family Zingiberaceae. Its polyphenolic compound curcuminoid is responsible for giving yellow color to the turmeric. Chemically curcumin has a methylene- 1, 3-diketo group that exhibiting keto-enol tautomerism. The possible antioxidant effects of curcumin against cerebral ischemia are reflected in various studies. Al omar *et al*. showed Curcumin remarkable effect on rat ischemic forebrain of B reduced MDA levels CCAO model. The protection rendered by Curcumin was due to increased activities of GSH, catalase and SOD and reduced MDA activity (Al Omar *et al*., 2006). Shukla *et al*. also evaluated the mechanism of Curcumin neuroprotection in MCAO induced focal cerebral IR injury in rats. The infarction and the cerebral edema was reduced, also high level of lipid peroxides in ipsilateral and contra lateral hemispheres of the brain observed after MCAO was reduced by curcumin

treatment. Curcumin also increased the SOD and GPx activities in the ipsilateral hemisphere suggesting that the nueroprotection was mediated through curcumin antioxidant activity (Shukla *et al*., 2008). Jiang *et al*. also investigated curcumin role against cerebral ischemia and found that curcumin significantly protected BBB integrity by reducing iNOS expression in astrocytes cells (Jiang *et al*., 2007).

Zhuang *et al***.** studied the anti-inflammatory effect of curcumin in gerbils induced with forebrain ischemia by BCCAO. Curcumin treated gerbils had decreased apoptotic neurons in the hippocampus CA1 region and the effect was due to decreased Jun and NF-κB expression (Zhuang *et al*., 2009).

Epigallocatechin-3-gallate (EGCG)

Catechins, a flavonoid chemical constituent obtained from fresh tea leaves of Camellia sinensis. The antioxidant properties of these constituents are linked with the increased level of transcription factors and antioxidant enzymes. EGCG is a polyphenolic compound abundantly found with broader pharmacological activity as anticancer, antioxidant, anti-inflammatory and cardioactive properties, because of which green tea polyphenols is recommended as possible neuroprotectant in progressive neurodegenerative disorders as they proficient to alter the brain revealed in various epidemiological studies. Wu *et al*. reported that EGCG prevented ROS generation by activation of Nrf2 that induced GST, GPX, GCL, HO-1 expression (Wu *et al*., 2006). A recent study has proved EGCG neuroprotective effect in gerbils induced with global ischemia by BCCAO. EGCG given at a dose of 25 or 50 mg/kg notably ameliorated hippocampal CA1 neuronal damage. Similar effects of EGCG were observed in a rat model of ischemia induced by MCAO, EGCG reduced infarction volume and modified the levels of MDA, glutathione and NO concentrations (Choi *et al*., 2004). Wei *et al*. conducted another study that revealed EGCG effects on modulating sodium nitroprusside (SNP) and NO concentrations as they were majorly involved in destroying the integrity of the BBB during ischemic conditions. EGCG attenuated the oxidative stress by reducing NADPH-d/nNOS expression (Wei *et al*., 2004). Jung *et al*. investigated EGCG effect on SNP-induced apoptosis in rat PC12 cells. EGCG proved to be anti-apoptotic by preventing mitochondrial release of cytochrome c into cytosol along with caspase-9 and caspase-3 inactivation (Jung *et al*., 2007).

Baicalin

Baicalin is the flavanoid compound obtained from plant Scutellaria baicalensi. It's a potent anti-inflammatory and anti-tumor agent and because of its wide range of pharmacological activity it is protective against focally induced cerebral ischemia-reperfusion injury in rats. Baicalin treatment significantly reduced infarct volume and via increased BDNF expression and reduced caspase-3 expression (Zhang *et al*., 2006). Tu *et al*. also found that baicalin reduced the neurological discrepancy scores and cerebral infarction in rats after pMCAO via significant decrease in the enzymatic activity of myeloperoxidase also with decreased expression of caspase-3 that ultimately inhibited apoptosis after pMCAO (Tu *et al*., 2012). The activation of several transcription factors generates various inflammatory reactions in cerebral ischemia-reperfusion. One of the receptors called NOD2 (nucleotide-binding oligomerization domain protein 2) involved in innate immune response is genetically linked with several inflammatory reactions. In this perspective, Li *et al*. also evaluated baicalin's effect on mice undergone with cerebral ischemia-reperfusion, Baicalin showed remarkable protection via down regulating the expression of NOD2 and TNFα that were up-regulated during oxygen-glucose deprivation, therefore revealing NOD2 as the target for baicalin, giving neural-protection on cerebral ischemia reperfusion injury (Li *et al*., 2010). Baicalin also provided protection in ischemia induced rat brain by MCAO via inhibiting TLR2/4 signaling pathway along with the reduction in the expression of TLR2/4, NFκB, COX-2 and iNOS in rat brain. The elevated level of NF-κB and p65 decreased significantly after baicalin treatment (Li *et al*., 2017). Another neuroprotective effect of baicalin was studied by Cao *et al*. in gerbils undergone with transient global cerebral ischemic-reperfusion injury where baicalin's treatment showed significant attenuation of neuronal cell damage brought up by ischemia and also brought significant elevation in the level of SOD, GSH and GSH-PX via promoting BDNF expression as well as inhibiting caspase-3. Thus all findings reveal that baicalin's neuroprotective effect may be allied with anti-oxidant and anti-apoptotic property (Cao *et al*., 2011).

Conclusion

Due to the complex pathophysiology of stroke and multifactorial effect of herbals, they may promise future for natural medicine for stroke. Their anti-oxidant, anti-apoptotic, anti-inflammatory and protective vascular effects are believed to be efficacious in treatment. For various neurodegenerative and chronic diseases, herbals have proved to be safer over longer period of time as they have fewer side-effects. Many active constituents of medicinal plants have shown neuroprotective effects when tested on laboratory bench but their transformation to clinical trials has been a huge challenge for stroke treatment. Hence, comprehensive data of safety and efficacy of medicinal plants should be generated and continuous efforts should be made to prove their effectiveness clinically. More consideration should be paid to natural compounds that can have extensive therapeutic time windows and that can prove to be perfect pharmacological targets with few side effects.

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