Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(2): 187-194
Received: 23-01-2016
Accepted: 25-02-2016

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Therapeutic role of phytoconstituents in treatment of cerebral ischemia/reperfusion injury

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Abstract
Stroke is important cause of mortality and morbidity in the world. Prevention and effective treatment of stroke is of utmost importance. Stroke involves reduce blood supply in brain to fulfill metabolic demand. Limit in number of medicines and their conventional target approach of action in treating stroke, led us to the search for novel therapeutic approaches. Higher extent of research in field of drug discovery focus on the possible ability of natural compounds extracted from medicinal plants, to prevent ischemia. Natural compounds are promising in treating stroke involving cell process like oxidative phosphorylation, membrane functioning, neurotransmitter release, and free radical generation. Herbal compounds show preventive effect in following sequence - calcium antagonism, anti-excitotoxicity, anti-oxidation, anti-apoptosis and anti-inflammation on experimental induced ischemic brain injury. In this review, we discussed cascade pathway of stroke, role medicinal compounds and novel medical pathway and hypothesis protecting brain ischemia or delay the neurological disorders following a stroke.

Keywords: stroke, anti-oxidation, anti-apoptosis, anti-excitotoxicity, anti-inflammation

Introduction
Stroke is a condition of lacking blood supply to the brain to meet needed energy demand which subjects brain to hypoxia and results to death of neurons or stroke. WHO characterizes stroke as rapidly progressing focal or global disturbance affecting brain roles, and symptoms lasts more than 24 h [1]. Recent survey suggests stroke as third largest cause of mortality after cancer and coronary heart disease and second largest cause of path physiological injury in adults [2]. The case of stroke is 1 per 1000 people [3] although; this incidence varies with age and sex. Arteriovascular diseases is one of the leading causes of death in developed countries [4] leading to neurorehabilitation [5]. Several *in-vitro* and *in-vivo* models of brain ischemia reported over the years. The *in-vitro* models include cultured neurons with or without synaptic formation, glia and cultured brain slice that could only suggest the cytotoxicity of the therapy. The *in-vivo* animal model of stroke reproduce the etiology, anatomical, and metabolic results of human pathology and allow the study of anti-ischemic drugs clinically proving therapeutic actions [6]. Animal models for stroke meant for screening of drugs classify in three subgroups as global ischemia, focal ischemia and forebrain ischemia (Table 1). Transient or permanent decrease of cerebral blood flow by thromboembolic or thromboembolic occlusion of artery focuses on thrombolytic therapy and is main rational therapeutic strategy for ischemic brain injury [7]. Reperfusion after thrombolytic therapy results in a series of cellular, biochemical and metabolic changes including intracellular reactive oxygen species (ROS) generation, calcium overload, excitotoxic cell injury and inflammation and finally contributes to irreversible brain injury. Tissue plasminogen activator preferred in treating early phase of cerebral ischemia [8].

Cascade pathway of cerebral ischemia
Various cell changes weaken the energetic actions that preserve ion gradients [9, 10]. Because of vascular occlusion brain tissue deprives of glucose and oxygen causing build-up of acidic by-products in cell [11, 12]. Decrease in glucose and pH level, lead to end of the electron transport chain within mitochondria causing sharp downfall of ATP and thus failure of energy homeostasis occurs [13, 14]. Deprivation of ATP causes disruption of ionic pump systems like Na^+-K^+-ATPase, Ca^{2+}-H^+ ATPase, Na^+-Ca^{2+} transporter resulting in intracellular increase of Na^+, Ca^{2+}, Cl^- concentration and efflux of K^+ [15, 16]. Redistribution of ions across plasma membrane causes neuronal depolarization, resulting in excess release of neurotransmitters, especially glutamate that causes neuronal excitotoxicity [17, 18].

Hyperactivation of glutamate on receptors like NMDA, AMPA and metabotropic causes increase in Ca^{2+} ion in nerve cells which trigger path physiological changes like necrosis and apoptosis [19, 20]. Changes include Ca^{2+} overload of mitochondria, generation of ROS, activation of enzymes like lipases, proteases, kinases that lead to necrosis. NO synthase activation, activation of caspases-9, 3, 8, bad, bax, & calpains result in oxidative stress, lipid peroxidation, inflammation and apoptosis [21, 22]. Ca^{2+} dependent activation of nNOS, increases NO production and formation of toxic $ONOO^-$ contributes to oxidative stress and excitotoxicity [23-25] (Chan PH 2001; Jordán J *et al.* 2008; Sharma SS 2003). Control of many enzyme like lipase, phosphatase, kinase, protease and endonuclease activate various inflammatory molecules like cytokine and interleukins (ILs) [26, 27] such as TNF-α, NF-κB causing neuroinflammation [28]. Accumulation of fluid occurs due to excessive influx of Na^+, Ca^{2+} ions and efflux of K^+ ions as well as action of inflammatory mediators like leukocytes [29, 30], adhesion molecule causing edema at site of injury [31, 32]. All above factors finally result in death of neuron cells and irreversible loss of neural role including cognitive roles [33, 34]. Cascade events of cerebral ischemia is described in Figure 1 and list of medicinal compounds exhibiting neuroprotective role is shown in Table (2).

Table 1

S No.	Experimental animal models	Subtypes
1.	Global ischemia	1. Total Body Ischemia Decapitation Cardiac arrest with resuscitation Profound systemic hypotension
		2. Global cerebral ischemia Increased intracranial pressure Combination of occlusion of the major arteries
2.	Forebrain ischemia	1. Bilateral common carotid occlusion in Mongolian gerbil hypotension. 2. Four vessel occlusion in the rats 3. Bilateral common carotid occlusion in spontaneously hypertension rats. 4. Two vessel occlusion with hypotension in rats.
3.	Focal ischemia	1. Middle cerebral artery occlusion Intraluminal arterial occlusion without craniotomy Mechanical or electrical arterial occlusion with craniotomy and dural opening 2. Photochemically induced focal cerebral thrombosis 3. Cerebral embolism Blood clot embolization Microsphere embolization

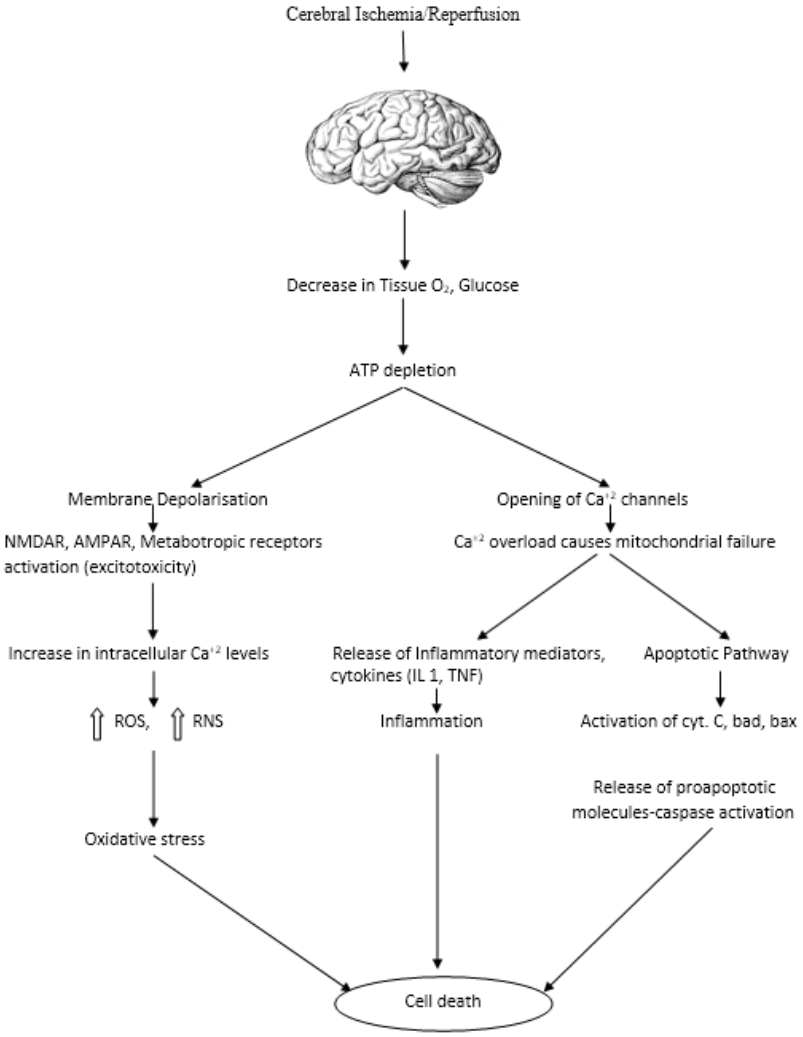

Fig 1: Pathway of cerebral ischemia

Table 2: Isolated medicinal compounds treating cerebral ischemia

Compounds	Origin	Dosage and route of exposure	*In vivo* and *in vitro* studies	Mechanism of action	Form of ischemia	References
Curcumin	Rhizome bioactive component of *Curcuma longa* Linn.	30 mg/kg (i.p)	Mongolian gerbils	Inhibition of mitochondrial mediated apoptosis	MCAO and BCAO	[35]
Scutellarin	Extracted from *Erigeron breviscapus*	50 and 75mg/kg (p.o.)	Male Sprague Dawley rats	Upregulation of eNOS and downregulation iNOS	MCAO	[36]
Ginkgolide B	Terpenic lactones present in *Ginkgo biloba*	5 mg/kg (i.v.)	Male and female tree shrews	Inhibiting pathological manifestation of PAF, such as inducing calcium overload, brain edema, and secondary brain damage in penumbra	Photochemically induced thrombotic cerebral ischemia	[37]
Crataegus flavanoids	Extracted from *Crataegus pinnatifida*	0.5 and 2.5 mg/ml orally	Male Mongolian gerbils	Scavenging effect on superoxide anion	MCAO and BCAO	[38]
Ascovertin	Complex of dihydroquercetin and ascorbic acid	70 mg/kg (p.o.)	Male and female wistar rats	Protection of brain Na^+ K^+ ATPase activity	LCAO	[39]
Safranal	Crocus sativus L. stigmas	72.5 mg/kg (i.p.)	Male NMRI rats	Antioxidant	Four vessel occlusion	[40]
SSF	A flavonoid isolated from aerial parts of Scutellaria baicalensis Georgi	35 mg/kg (p.o.)	SD rats	Block the pathogenic process in vascular dementia ameliorating cognitive deficit protecting neuronal injury and modulating abnormal changes in energy metabolites	BCAO	[41]
Lecithin and α tocopherol	Structural elements of biological membrane	Lecithin 300 mg/kg, and tocopherol 200 mg/kg(p.o.)	Male wistar rats	Antioxidant	BCAO	[42]
Thymoquinone	Nigella sativa seeds oil	10 mg/kg and 2.5, 5, and 10 mg/kg (i.p.)	NMRI rats	Inhibition of lipids peroxidation	Four vessel occlusion method	[43, 44]
Buckwheat polyphenol	Polyphenolic compounds such as catechin, epicatechin, quercetin, and epicatechin from Fagopyrum esculentum	600 mg/kg (p.o.)	Male wistar rats	Inhibition of excess release of glutamate Inhibiton of delayed generation of NO_2	Repeated cerebral ischemia(10 min., two times occlusion, 1 h interval)	[45]

MCAO- middle cerebral artery occlusion; BCAO-bilateral common carotid artery occlusion; LCAO- left common carotid artery occlusion; eNOS- endothelial nitric oxide synthase; iNOS- inducible nitric oxide synthase; PAF- platelet activating factor; NMRI-naked mole rat initiative, SD-Sprague Dawley.

Natural compounds with calcium antagonization effects

ROS bursts and excitatory glutamate toxicity because of ischemia/ reperfusion results in intracellular Ca^{2+} overload. Excess concentration of Ca^{2+} in neuron begins disastrous events leading to irreversible neuronal injury. Natural compounds like Guattegaumerine (bisbenzylisoquinoline alkaloid obtained from *Guatteria gaumeri*) [46] and tetrahydroxystilbene glucoside (TSG) [47], have shown promising results by reducing ischemia induced Ca^{2+} overload in neurons. VGCC blockers are significant therapeutic approach for post-ischemia neuroprotection in humans. Research studies have shown that some isoquinoline alkaloids extracted such as berberine (an alkaloid derived from *Rhizoma coptidis*) and palmatine (a flavonoid in *propolis*) show rapid inhibition of voltage-gated calcium entry in many native cells [48, 49].

Natural compounds with anti-excitotoxicity properties

Enormous release of excitatory neurotransmitters, particularly glutamate in synaptic cleft causes over activation of NMDA and AMPA receptors that leads to influx of calcium and afterwards disturb ionic homeostasis. As a result cytotoxic edema and oxidative stress occur and results in cellular damage. Increased calcium levels activate enzymes such as lipase, protease, and endonuclease that may damage DNA, cell proteins, and lipids thus leading to cellular necrosis [50, 51]. Some medicinal plant compounds have shown neuroprotective by reducing glutamate release, inhibiting glutamate receptor stimulation, or reducing cellular Ca^{2+} overload [40, 52] (Hosseinzadeh H *et al.* 2005; Lee JM *et al.* 2000). Example medicinal extracts of *Opuntia ficus-indica (Cactaceae)* and *Alpinae oxyphyllae (Zingiberaceae)* showed neuroprotective effect against NMDA-induced and KA induced neurotoxicity in cultured neuronal cells [53, 54].

Natural compounds with anti-oxidative properties

A research has reported that a burst of free radical oxygen species like ROS produce during ischemia or reperfusion, which later leads to the lipid oxidation, damage to proteins and DNA and apoptosis [55, 56]. Natural compounds showing antioxidant effect includes flavonoids obtained from *Scutellaria baicalensis* Georgi [57], CA present in the herb rosemary obtained from *Rosmarinus officinalis* [58], Curcuma Oil (isolated from powdered rhizomes of *Curcuma longa*) [59], *Ginkgo biloba* extract EGb761 [60], and Cinnamophilin (isolated from *Cinnamomum philippinense*) [61]. In a scientific study pretreatment with curcuma oil, extracted from powdered rhizomes of *Curcuma longa* Linn, substantially reduces the levels of nitric oxide, reactive oxygen species and peroxynitrite and regulates the mitochondrial membrane potential [59]. Certain plant compounds like saponin and tannins extracted from root of *Salvia leriifolia Benth* (Lamiaceae) and seed oil of *Nigella sativa* (Ranunculaceae) has shown important anti-oxidative role by inhibiting lipid peroxidation. Hence reducing the levels of MDA and later improving the overall anti-oxidative mechanism by increasing the levels of superoxide dismutase, catalase, glutathione

peroxidase, and decreasing the level of reactive nitrogen species and reactive oxygen species [43, 62].

Natural compounds showed therapeutic effects on endogenous antioxidant enzyme levels like carnosic acid obtained from *Rosmarinus officinalis* induces haeme oxygenase-1. Heme oxygenase (HO) is the rate-limiting enzyme for catabolism of heme, a process that gives bile pigment biliverdin (antioxidant), iron and carbon monoxide. Heme oxygenase exists in two forms- an inducible form (HO-1) and a constitutively expressed form (HO-2). HO-1 induces in response to noxious stimuli like hypoxia and oxidative stress and protect against I/R injury [63-65]. The cytoplasmic enzyme NADPH oxidase is responsible for ROS production in cerebral ischemia and has gained a lot of focus in recent years. Over activation of the neuronal *N*-methyl-*D*-aspartate receptor (NMDAR) causes superoxide generation and causes neuronal death. Recent study suggests activation of NADPH oxidase for NMDAR-mediated superoxide generation (Brennan AM 2009). Certain natural compounds like sinomenine extracted from *Sinomenium acutum* inhibit activation of microglial NADPH oxidase [66].

Natural compounds with anti-apoptotic effects

Two general pathways in general regulate the cerebral ischemia- intrinsic and extrinsic pathways. Intrinsic pathway originates from mitochondrial release of cytochrome c and resulting stimulation of caspase-3. Increased intracellular calcium activates Bid (tBid) which interacts with apoptotic proteins such as Bad and Bax, on the mitochondrial membrane that leads to release of cytochrome c (Cytc) which further binds to apoptotic protein-activating factor-1(APAF) and procaspase-9 that activates caspase-9 and caspase-3. Activated caspase3 damage deoxyribonucleic acid and cause apoptosis [67, 68]. In extrinsic pathway, the extracellular Fas ligand binds to Fas death receptors and strengthens caspase-8 activation which leads to apoptotic cascade [67]. The death receptor Fas belongs to the tumor necrosis factor (TNF) receptor superfamily, and regulates death and survival of cells, as well as growth and differentiation [69, 14]. Fas ligand (Fas-L) activates Fas in an autocrine or paracrine pattern, which causes trimerization of Fas with Fas-associating proteins with death domain (FADD) and procaspase-8. Fas stimulation contributes to cascade of actions contribute to apoptosis of neuronal cells [70-72]. Certain herbal compounds have inhibitory effect on intrinsic or extrinsic pathways of apoptosis. Curcumin isolated from the rhizome of *Curcuma longa Linn* (Zingiberaceae) inhibit mitochondrial-mediated apoptotic signaling pathway [35].

There lies a physical balance between anti-apoptotic and pro-apoptotic members of the Bcl-2 family, imbalance of which controls the survival and death of developing and mature cells. The pro-apoptotic members of Bcl-2 family include Bax, Bcl-xs, Bak, Bad, Bid and these plays an important role in ischemic neuronal death [73]. Anti-apoptotic members of Bcl-2 family include Bcl-xL, and Bcl-w. Excessive expression of Bcl-xL protein in the adult brain suppresses activation of procaspase 9 by forming a complex with Apaf1 and thus prevents the release of cytochrome c from mitochondria, and maintains cell viability. A number of herbal drugs have shown their action on anti-apoptotic pathways, such as Bcl-2 family proteins. Therefore in recent researches Bcl-xL has become a promising target for drugs to reduce cell apoptosis [74]. Administration of 4-hydroxybenzyl alcohol (an active phenol constituents of *Gastrodia elata Blume)* 30 min. before ischemia antagonize cerebral ischemia by increasing Bcl-2 expression and inhibiting caspase-3 activity, ameliorate cell apoptosis in ischemic regions [75].

Natural compounds with anti-inflammatory effects

Microglial activation, perivascular and parenchymal macrophages, infiltration of peripheral inflammatory cells and expression of cytokines, adhesion molecules, chemokines, and leukocytes occurs few hours of ischemia or reperfusion injury. Research studies suggest the role of neutrophils in inducing the ischemic cerebral damage; reduction and decreased infiltration of neutrophils ameliorate the ischemic brain injury [76]. There are many biologically active moieties that prove antiinflammatory and neuroprotective effects against ischemia e.g. *Uncaria rhynchophylla (Rubiaceae)* extract exhibits neuroprotective effect by inhibiting cyclooxygenase-2 within hippocampus and *in-vitro* inhibition of proinflammatory process like microglial activation [77].

Novel pathways regulating neuroprotection

A major polyphenol component of green tea EGCG, improve age-related cognitive decline and protect against cerebral ischemia or reperfusion injuries [78, 79] and brain inflammation and neuronal damage in experimental autoimmune encephalomyelitis [80]. Several evidences suggest the biological action of green tea catechins is not only because of their antioxidant or radical-scavenging potential but also because of modulating various protein kinase signaling pathways. *In vitro* cell-signaling studies on the neuroprotective action of EGCG revealed a specific involvement of protein kinase C [81, 82], a family of serine/threonine kinases consisting of 11 isoforms, and divided into subclass: conventional (α, β_1, β_2, γ), novel (δ, ε, μ, η, Θ) [83]. Induced PKC in neurons is neuroprotective against several exogenous insults. Indeed, PKCε activation after ischemic preconditioning or pharmacological preconditioning (with either PKCε, NMDA, or A1AR agonists) showed necessary for neuroprotection against oxygen/glucose deprivation in organotypic slice cultures [84]. Activation of EGCG by PKC prevents apoptosis and mitochondrial membrane collapse. Rapid phosphorylative activation of PKC by EGCG accounts for its neuroprotective activity against several neurotoxins such as A β (28), 6-OHDA [81].

A novel pathway in the neuroprotective mechanism of action of EGCG involves a rapid PKC mediated degradation of the Bad protein by the ubiquitin proteasome system and pronounced reduction after 24 h in cell culture [82]. Bad protein is responsible for opening of the mitochondrial mega channel permeability transition pore by its heterodimerization with the mitochondrial death suppressor proteins Bcl-2 and Bcl xL, thus neutralizing their anti-apoptotic role [85]. Administration of EGCG for 30 min prevented scattering the mitochondrial membrane potential, induced by short-term (4 h) exposure to 6-OHDA. Apart from PKC, other cell signaling pathways have been implicated in the action of green tea catechins, such as the mitogen-activated protein kinases (MAPK), phosphatidylinositide 3'-OH kinase/AKT and protein kinase A signaling cascades, and cell calcium influx regulation [86]. These cascades have shown central roles in neuronal protection against various extracellular insults [87, 88].

Novel medical hypothesis

It is imperative that ischemia or reperfusion cascade begins

with disruption of cerebral blood flow that deprives brain cells of glucose and oxygen supply, resulting in decrease in energy production and accumulation of toxic substances like glutamate, inflammatory mediators, and free radicals that could eventually result in neurodegeneration [89]. Oxidative damage plays a key role during ischemia. Neuroprotective role of Nrf2 can is highlighted by up-regulating a series of ARE driven anti-oxidative and cytoprotective genes to defend against oxidative stress [90]. Hence, agents that interfere with the Nrf2 pathway show a neuroprotective potential. AKBA, a novel Nrf2 activator, enhance the protective defense mechanisms through the HO-1 neuroprotective pathway. Nrf2 is a key regulator of endogenous antioxidant defense. Nrf2 remained bound to its cytosolic inhibitor, Kelch-like ECH-associated protein 1 (Keap1), and remains in the cytoplasm before targeted for proteosomal degradation [91]. Activation and nuclear accumulation of Nrf2 influences endogenous antioxidant defenses to restore cellular redox homeostasis by inducting phase II defense enzymes.

Various defense mechanisms activate Nrf2 and increases expression of antioxidative genes. AKBA is a potent antioxidant in treating oxidative injuries [92]. In a study AKBA showed protective effect against cerebral I/R injury in MCAO model proved by improved neurologic condition, reduced infarct volume and ameliorated neuronal apoptosis. The mechanism involved is by activating Nrf2/HO-1 pathway. Recently, triterpenoids such as maslinic acid and oleanolic acid being structurally similar to AKBA, reported significant increase in Nrf2, promising neuroprotection [93, 94].

The mitochondrial presence of nearly 1,000 different proteins, two different membranes, many transporters for metabolites, proteins, and ions provide target for both injury and cytoprotection. Similar to injured cells, damaged mitochondria can destroy their immediate environment, leading to the death of the host cells where they remain. Various tactics for protecting cells against pathologic mitochondrial injury includes (1) optimization of mitochondrial dynamics through mitophagic disposal of abnormal mitochondria and biogenesis of new, healthy mitochondria. (2) Use of ischemic-preconditioning mechanisms like mitochondrial protein phosphorylation and sirtuin activation that improve mitochondrial bioenergetics. (3) Inhibition of mitochondrial oxidative stress by inhibition of mitochondrial superoxide production, by mitochondrially targeted antioxidants, and by stimulation of antioxidant gene expression that increases endogenous mitochondrial antioxidant defenses. Medicinal compounds or conditions that activate all three pathways show neuroprotection [95].

Abbreviations

1.	A1AR	A 1 Adenosine Receptor
2.	AMPA	α-Amino 3 Hydroxy 5 Methyl 4 Isoxazolepropionic Acid
3.	AKBA	Acetyl 11 Keto Beta Boswellic Acid
4.	APAF	Apoptotic Protein Activating Factor 1
5.	ARE	Antioxidant Response Element
6.	ATP	Adenosine Triphosphate
7.	BBB	Blood Brain Barrier
8.	CA	Carnosic Acid
9.	Cyt c	Cytochrome C
10.	DNA	Deoxy Ribose Nucleic Acid
11.	EGCG	2 Epigallocatechin 3 Gallate
12.	FADD	Fas Associating Proteins With Death Domain
13.	HO	Heme Oxygenase
14.	ILs	Interleukins
15.	I/R	Ischemia/Reperfusion
16.	KA	Kainic Acid
17.	Keap 1	Kelch Like ECH Associated Protein 1
18.	MAPK	Mitogen Activated Protein Kinase
19.	MDA	Malondialdehyde
20.	NADPH	Nicotinamide Adenine Dinucleotide Phosphate Oxidase
21.	NF-κB	Nuclear Factor Kappa Light Chain Enhancer of Activated B Cells
22.	NMDA	N Methyl D Aspartate
23.	NMDAR	N Methyl D Aspartate Receptor
24.	NO	Nitric Oxide
25.	nNOS	Neuronal Nitric Oxide Synthase
26.	6-OHDA	6-Hydroxydopamine
27.	$ONOO^-$	Peroxynitrite
28.	PKC	Protein Kinase C
29.	ROS	Reactive Oxygen Species
30.	TNF-α	Tumor Necrosis Factor-α
31.	TSG	Tetrahydroxystilbene Glucoside
32.	VGCC	Voltage Gated Calcium Channel
33.	WHO	World Health Organization

Conclusion

Herbal compounds have vast therapeutic potential and used as a treatment measure in cerebral ischemia. Because of availability, lower cost, and fewer adverse affects of herbal compounds in comparison to synthetic makes them as an excellent choice in treating stroke. Cerebral ischemia involves multifactorial progressive path physiological events which suggest designing cocktail drugs that target different signaling events entering cerebral ischemic damage. Therapeutic combination of herbal medicines and neurochemical agents could be a better blend in ameliorating cerebral ischemia in patients. Preclinical and clinical trials will ensure the combination therapy as a good alternative for treating cerebral ischemia in humans.

Acknowledgments-None

Conflict of interests - The authors declares there are no conflicts of interests.

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