CHAPTER 2

REVIEW OF LITERATURE

Chapter Highlights

- ***Cerebral ischemia pathophysiology***
- ***Therapeutic approaches in cerebral ischemia***
- ***An overview of neuroprotection***
- ***Withanolide A***
- ***Role of endogenous hormones in neuroprotection***
- ***Rodent models for study of ischemic pathophysiology***
- ***Virtual screening of inhibitors***

2.1 Pathophysiology of Cerebral ischemia

Cerebral ischemia reperfusion injury (CIRI) continues to be a major contributor in the global map of diseases and disorders and remains the second leading cause of death and the third most debilitating neurological disorder worldwide [1]. Cerebral ischemia is commonly caused due to compromised blood flow in a brain region as a result of an occluded blood vessel often causing neurological deficits and formation of infarction or lesions in the damaged area [3]. The infarct region witnesses rapid cell death, severe fall of ATP levels and energy reservoirs of cell and experiences a major imbalance of ionic homeostasis [3]. The serious nature of damage in the core of the infarct region is often irreversible, even after restoration of cerebral blood flow [3]. A multitude of biochemical and molecular processes like glutamate excitotoxicity, ionic imbalance, free radical generation, etc., causes loss of cellular integrity and hampers neurologic functions [4]. These myriad of biochemical changes causes DNA

damage, which finally leads to activation of various caspase dependent and independent cell death pathways [3] (Fig. 2.1.). The induction of DNA breaks due to excessive nitrosative and oxidative stress during cerebral ischemia, causes activation of PARP-1 enzyme [3], which causes cell death through an energy failure mechanism [5]. PARP-1 mediated cell death is often termed as "PARP-1 suicide hypothesis" which states that over activation of PARP-1 due to DNA damage is responsible for depletion of its substrate NAD, finally leading to cell death by energy failure [5]. The PARP-1 dependent pathway is also termed as Parthanatos and is independent of caspase mediated cell death, a hallmark of apoptosis [6]. PARP-1 activation is followed by mitochondrial disintegration, translocation of apoptosis inducing factor (AIF) from mitochondria to nucleus and loss of cellular ATP and NAD reservoir [6]. Caspases do not appear to play major role in Parthanatos, since broad spectrum caspase inhibitors were unable to confer protection in PARP-1 mediated cell death [6]. Considering the role PARP-1 plays in cerebral ischemia, several studies have inferred that targeting PARP-1 could be a possible therapeutic strategy [3, 5].

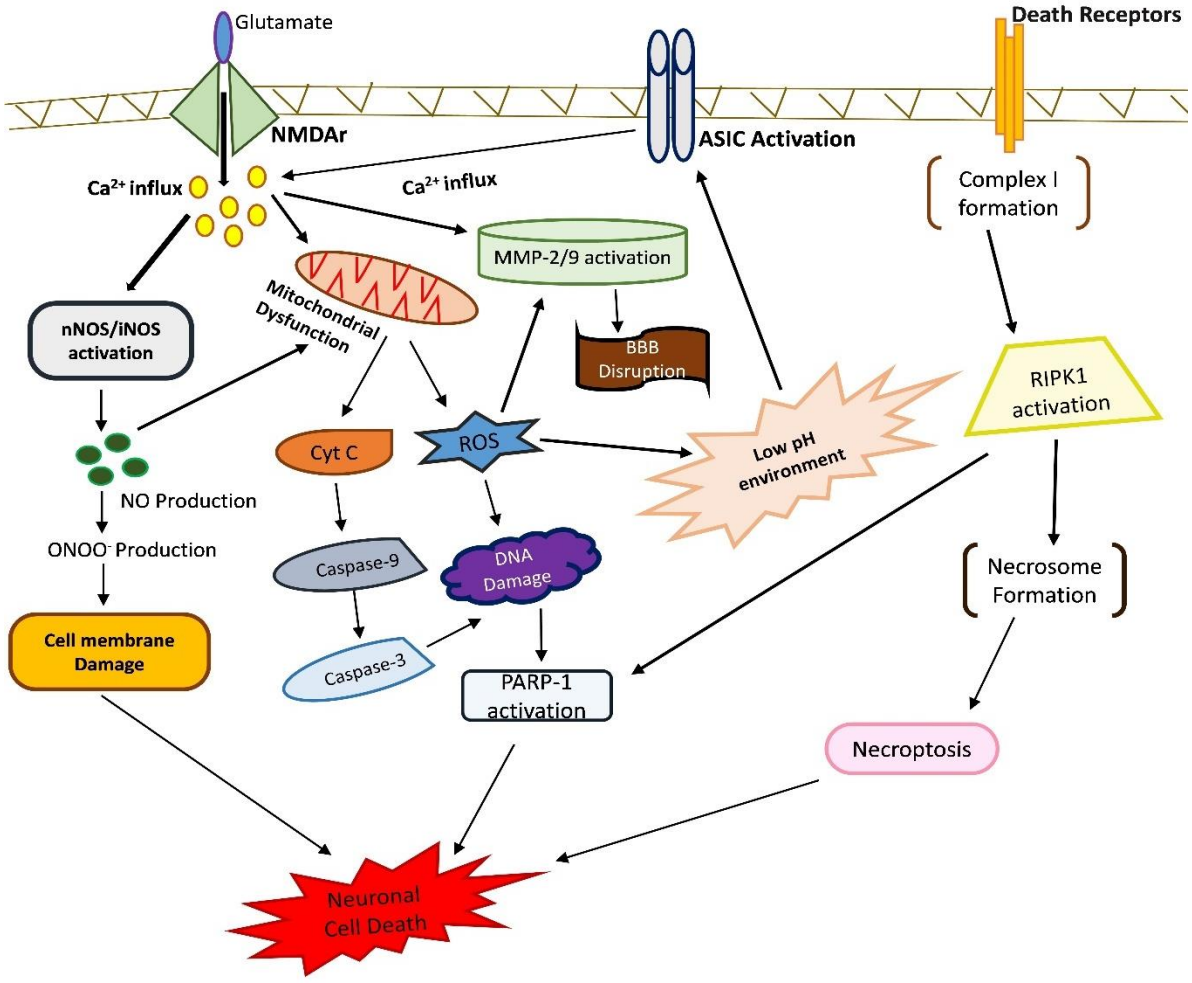

Fig. 2.1. Cerebral ischemia pathophysiological cascade

Among the myriad processes contributing to neuronal death in cerebral ischemia, another caspase independent pathway, has been identified to play prominent importance [7]. Among the principal cell death pathways, apoptosis and necrosis has always been significant. Though apoptosis is a programmed cell death pathway, necrosis is generally known as an unregulated process. But recent studies have established a programmed necrotic cell death pathway, termed as Necroptosis, which is involved in ischemic injury and is induced by activation by death-domain receptors (DRs) and is devoid of caspase signaling [7]. Execution of the pathway depends on interaction of RIPK1 with receptor interacting protein kinase 3 (RIP3) [8] and inhibition of necroptosis by specific inhibitors, known as necrostatins, has ameliorated

ischemia-reperfusion injury induced pathophysiological conditions in rodent model [7]. Necrostatins have been later characterized as specific inhibitors of RIPK1 [9], which indicates major involvement of RIPK1 in necroptotic pathway and that inhibition of RIPK1 might be successful approach to combat ischemia induced brain damage. In addition to being the major mediator of necroptotic pathway, a recent study has identified another major role RIPK1 plays in Parthanatos [10]. Regardless of its kinase activity, nuclear RIPK1 activates PARP-1 under oxidative stress condition, thus establishing itself as indispensable in two major caspase independent cell death pathways [10]. Oxidative stress is a common characteristic observed in cerebral ischemia pathophysiology, hence, RIPK1 might be key modulator in ischemic cell death and thereby an important therapeutic target. Identifying of inhibitors against both RIPK1 and PARP-1 could be a suitable approach towards future designing of potent neurotherapeutics.

2.2. Therapeutics in cerebral ischemia

Identification of symptoms of cerebral ischemia goes down a long way in history of mankind, with the first documentation of the disorder by Hippocrates in his scrolls during 460 to 370 B.C., when he described a condition of onset of paralysis due to wound in brain as apoplexy, which in recent scientific world is termed as transient ischemic attack (TIA) [11]. Galen (AD 131 to 201), did put forth the concept of presence of brain lesion, but was unable to satisfactorily explain the reason behind apoplexy [11]. After Galen's theory was proposed, for several centuries European medicine remained in dark regarding nature and character of apoplexy or ischemic attack [11]. With the advancement of science and medicine, various therapeutic strategies have emerged for treatment of cerebral ischemia induced injury.

2.2.1. Treatment with Thrombolytic Drugs

Intravenous administration of recombinant tissue Plasminogen Activator (rtPA) for treatment of cerebral ischemia was approved by FDA in 1987 which proved effective but with the limitation of administration within a stipulated time duration of 3 hours of onset of the symptoms [12]. Efficacy of rtPA (commonly marketed as Alteplase), decreases notably after 4.5 hours of inception of ischemic insult [13]. Alteplase is the only FDA approved thrombolytic drug from cerebral ischemia, whereas its contemporary thrombolytic drugs streptokinase and urokinase, did not succeed the clinical trials [12]. The efficiency of rtPA in early reperfusion makes it a therapy of choice, but this same property often contributes to the most serious limitation associated with rtPA, which is intra cranial hemorrhage (ICH) [13]. The hemorrhage is often observed in the area of infarction and can often be fatal [13].

2.2.2. Endovascular Thrombectomy Strategy

Endovascular thrombectomy or EVT is a mechanical technique for clot retrieval from occluded arteries which has been reported to provide efficient reperfusion rates as compared to rtPA [14-16]. The process involves use of micro catheters and stent retrievers to retrieve the clots from occlusion sites and angiographic imaging is used to guide the tools through the vascular tree [12]. The thrombus or embolus is either aspirated by stent retrievers delivered via micro catheters or by using a large suction catheter [12]. Though reported as effective treatment strategy and is mandatory for patients with large vessel occlusion (LVO), a very recent multi-center cohort study revealed that patients with suffering from mild cerebral ischemic damage due to LVO who received EVT as treatment, were at no excellent benefit as compared to those receiving medical management [17].

2.2.3. Antiplatelets and Anticoagulants

Use of antiplatelet drugs to reduce aggregation of platelets, thereby lowering chances of clot formation might be an effective strategy for prevention of cerebral ischemia. Various antiplatelet compounds are presently in phase III and IV trials but use of aspirin for treating early stage of ischemic attack is a widely accepted strategy [18]. Two other antiplatelet agents, clopidogrel and ticlopidine are usually used to treat aspirin intolerant patients, but these drugs are not as efficient as aspirin and may have toxic side effects [19]. Another well studied strategy for treatment of cerebral ischemia is use of anticoagulants, which inhibits blood clotting by blocking the vitamin K-dependent clotting factor production. Commonly used anticoagulants are different thrombin inhibitors, heparins and heparinoids [20]. Anticoagulants have not succeeded clinical trials since their administration poses threat of excess bleeding, ICH and thereby prove fatal.

2.2.4. Neuroprotective Strategies

Apart from the "vascular approaches" of therapeutic strategies to combat cerebral ischemia reperfusion injury, which act by restoring the cerebral blood flow by removal or dissolution of clot, another widely studied method of ameliorating ischemia-induced neural damage can be termed as "cellular approach" [21]. This approach is also defined as neuroprotection, which involves strategies aiming at antagonizing the damage induced by cerebral ischemia and thereby allowing survival of brain cells [22, 23]. Neuroprotection strategies first emerged during the 1970s and started developing during the 2000s [24]. During the 1990s, extensive scientific studies started revealing the underlying cellular and molecular mechanisms involved in ischemic insult and cerebral tissue damage, which provided an understanding regarding the relevant molecular mediators of ischemic cascade which can be pharmacologically targeted

[25]. Intense research regarding the excitotoxicity caused as a result of lack of blood flow and energy loss during ischemic conditions established the roles of N-methyl-d-aspartate receptor (NMDAr) signaling, excitatory amino acids and involvement of calcium channels in neuronal death acceleration [22]. Several neuroprotective strategies were devised by targeting these molecules, but most them failed to succeed the clinical trials [26].

Beside the excitotoxic pathway, the surge in oxidative stress and inflammatory responses led to the idea that cerebral ischemia is not only a vascular disease, but it also involves various neuronal and vascular cells like neurons, astroglia, macrophages, etc., which contributes to the cellular and molecular cascades of ischemic pathophysiology [24]. These findings led to establishment of the concept of neurovascular units, involving the neurons, endothelial cells and astrocytes and was later expanded to encompass the roles of perivascular nerves, smooth muscles cells and venous systems in cerebrovascular damages [27]. Though the concept of neurovascular unit was proposed in 2002, till date, no drug targeting the neurovascular units has been clinically approved [24].

By the late 1990s and early 2000s, role of PARP-1 activation was linked with cerebral ischemia and its properties like kinase-dependent and Ca^{2+} dependent activation might be of high importance [28-31]. Over activation of PARP-1 during cerebral ischemia causes derangement of neurovascular unit, which is prompted by its ability to cause mitochondrial dysfunction, increase expression of matrix metalloproteinases (MMPs) thereby disrupting BBB integrity and exhausting cellular energy store, finally causing neuronal death [21]. The role of PARP-1 in deranging the integrity of neurovascular unit gives an opportunity for designing PARP-1 inhibitors as potential neuroprotectants. Several studies have reported that PARP-1 inhibition by PJ34 and 3-aminobenzamide ameliorates cerebral ischemic pathophysiology [32, 33].

Early 2000s saw the discovery of a cell death pathway completely different to apoptosis and necrosis, which were till date considered as primary cellular death pathways [34], and was termed as necroptosis, a programmed necrotic cell death [7]. Necroptosis has been now established as a cell death component of various neurodegenerative diseases like Alzheimer's disease [35, 36], Parkinson disease [37], Huntington disease [38], Amyotrophic lateral sclerosis [39, 40], Gaucher's disease [41] and cerebral ischemia [7]. Recent evidences suggest that inhibition of necroptosis can be a suitable strategy to combat ischemia induced neuro-degeneration and suitable inhibitors of necroptosis needs to be identified. Though the reported RIPK1 inhibitor Nec-1 inhibits necroptosis is BBB permeable, short half-life of 1hr renders it unsuitable for treating chronic diseases [42]. RIPK1 remains a potent pharmacological target for cerebral ischemia till date, inhibitors which might prove successful neuroprotective agents against cerebral ischemia.

During the past decade, there have been a significant increase of interest in use of phytochemicals as neuroprotectants. Numerous scientific studies have proved anti-inflammatory and anti-oxidant properties of compounds present in various medicinal herbs which can be exploited to design potent neuroprotectors against brain pathologies [Table 2.1].

Table 2.1.: Phytochemicals and their neuroprotective properties

Phytochemicals	Source	Neuroprotective Effects
Resveratrol	Berries, peanuts, grapes and medicinal herbs [43]	Reduces oxidative stress by decreasing production of ROS and superoxide ions. [44, 45]. Prevents neuronal death from β-amyloid (Aβ) induced toxicity [46].
Curcuminoids	*Curcuma longa*	Effective in suppression of microglial inflammatory responses and reduction of amyloid plaques in Alzheimer's disease [47,48]. Reduces pathogenesis in Parkinson's disease by controlling the oxidative and inflammatory pathways [49].
Epigallocatechin-3-gallate	Green tea	Ameliorates neural damage by reducing oxidative stress and neuroinflammation [50, 51].
Caffeic acid phenethyl ester	Honeybee propolis	Protects neurons from inflammatory and oxidative stress by inhibiting NF-κB signaling [52,53]. Prevents dopaminergic neuron loss [54].

Ginsenosides	Ginseng	Attenuates brain excitotoxicity, reduces neuroinflammation, prevents apoptosis and restores neurotransmitter levels [55-57]. Modulates the levels of cytokines TNF-α and IL-1 and thus reduces α-synuclein-mediated neuroinflammation [58]
Berberine	*Berberis vulgaris*, *Berberis aristata*	Checks progression of Alzheimer's disease [59]. Acts as a ROS scavenger and inhibits apoptosis mediators, thereby checking neuronal death [60].
Withanolides	*Withania somnifera*	Controls oxidative stress and lipid peroxidation induced damage [61].

Therapeutics against cerebral ischemia requires identification of novel compounds for prevention of neurological damage caused by ischemic excitotoxicity. The lack of toxicity and ability to intervene the cellular and molecular pathways involved in ischemic cell death, establishes phytochemicals as an apt choice for neurotherapeutics, but further studies are still necessary for clinical use of these molecules.

2.3. Withanolide A as a neuroprotectant

Withanolide A (WA) (Fig. 2.2.) is a steroidal lactone and a major constituent of the root of an Indian Ayurvedic herb Aswagandha (*Withania somnifera*) [62]. WA has been reported to

confer neuroprotection in Alzheimer's disease by ameliorating amyloid pathologies [63]. WA also induces neurite regeneration, promotes axonal outgrowth of cortical neurons and restores synaptic damage [64,65]. A recent study also reports that WA augments stress resistance and prolongs longevity in *C. elegans* [66]. During hypobaric hypoxia conditions, WA exerts neuroprotection in hippocampal neurons by enhancing biosynthesis of endogenous glutathione via Nrf2 pathway [67].

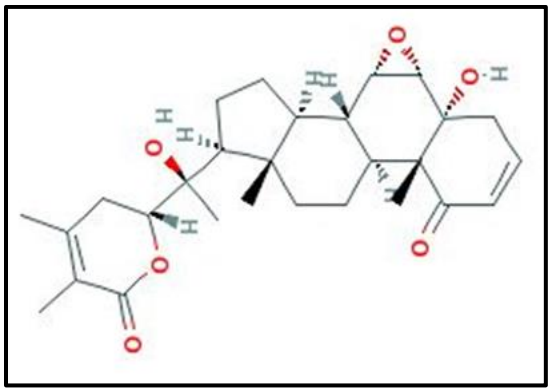

Fig. 2.2. Chemical Structure of Withanolide A

The neuroprotective qualities exhibited by WA makes the phytochemical a molecule of interest in therapeutic research for cerebral ischemic injury. But, till date effect of WA in *in-vivo* cerebral ischemia has not been explored.

2.4. Endogenous hormones and Neuroprotection

Various studies have suggested a gender dependent variation in cerebrovascular events including brain injury due to hypoxia [68], drug induced excitotoxicity [69] or cerebral contusion [70]. Even in experimental stroke models, smaller cerebral infarcts are detected in female rats as compared to rodents of opposite sex of the same age group [71]. Similar kind of results were observed in diabetes and hypertension linked ischemic models [71, 72]. These

observations have led to investigation of the role of the female sex hormones in conferring neuroprotection and estrogen has been often linked with this phenomenon. Estrogen group of hormones consists of four molecules, namely, Estrone (E1), Estradiol (E2), Estriol (E3) and Estetrol (E4) (Fig. 2.3.) [73].

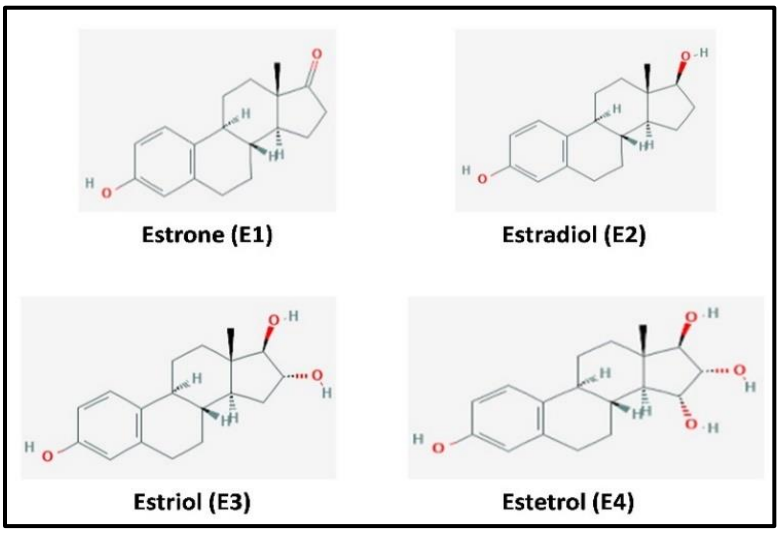

Fig. 2.3. Structure of Estrogen Hormones

Several studies have reported that administration of E2, in ovariectomized females in experimental models of cerebral ischemia, exhibits profound neuroprotective effects [74-76]. Several *in-vitro* and *in-vivo* studies have associated E2 mediated neuroprotection with its ability to up regulate Bcl-2 expression and activate the PI3K/ AKT pathway, which is necessary for cell survival [77-80]. E2 also reduces post-ischemic inflammation by lowering expression of interleukin 1- β (IL-1β) and TNFα, thereby suppressing matrix metallopeptidase 9 (MMP-9), thus exerting its anti-inflammatory properties to reduce brain injury [81, 82]. E3 has been reported as a neuroprotective agent in autoimmune encephalomyelitis (EAE), an experimental mouse model for studying chronic form of human relapsing-remitting Multiple Sclerosis [83]. E3 exerts neuroprotective effect by increasing the production of cytokines IL-

10 and IL-5 [84, 85] and works in a gender unspecified manner [83]. Though very little has been reported till date about neuroprotective ability of E4, a recent study confirms its neuroprotective ability in neonatal hypoxic–ischemic encephalopathy (nHIE) in rat model [86]. Neuroprotection by estrogens are reportedly mediated by the estrogen receptors (ERs) present in the cortical regions, neurons and endothelial cells [87]. Progesterone (Fig. 2.4.a) is another reproductive hormone that has been studied extensively in last decade for its neuroprotective ability in cerebral ischemia [87]. Progesterone reduces ischemia induced inflammation by decreasing the production of IL-1β [88], TNF-α [89], and TGF-β [88] and by reducing oxidative stress by inducing production of antioxidants in brain [89]. The anti-inflammatory property of progesterone also contributes to its ability to reduce cerebral edema induced by ischemic damage [88, 90,91]. It has been also theorized that, metabolism of progesterone to allopregnanolone (Fig. 2.4.b) confers protection against ischemia induced excitotoxicity by preventing down regulation of $GABA_A$ receptors [87].

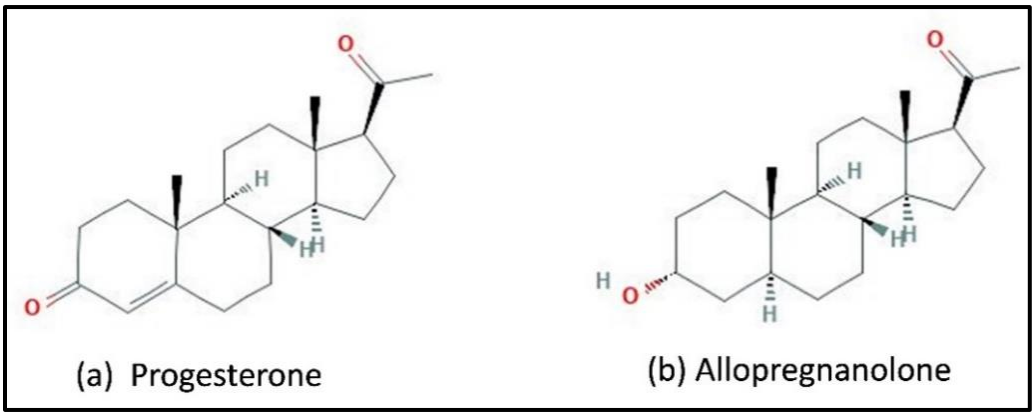

Fig. 2.4.: Chemical Structures of (a) Progesterone and (b) Allopregnanolone

Prolactin (PRL) (Fig. 2.5.) is a peptide hormone synthesized by the lactotrophs present in the anterior pituitary gland [92] and is mainly associated with maternal behavior and lactation [93]. Besides its role in milk secretion, PRL exhibits it versatility brain by participating in glial

activation [94], stimulating regeneration of neurons in olfactory bulb [95], proliferation of hippocampal precursor cells [96], modulation of oligodendrocyte remyelination [97] and repairing of white matter damage [98]. Various *in-vitro* and *in-vivo* studies also exhibit neuroprotective ability of PRL against glutamate and kainic acid induced excitotoxicity [99-101]. PRL shows neuroprotective effect against glutamate excitotoxicity by lowering calcium ion concentration (which increases as a result of excitotoxic insult) and modulating NF-κβ pathway to up regulate expression of Bcl-2 protein, which is necessary for cell survival [102]. Though scientific studies have widely established neuroprotective role of PRL and has been trying to decipher the underlying mechanisms, *in-vivo* efficacy of PRL in combating cerebral ischemic pathophysiology is yet to be reported.

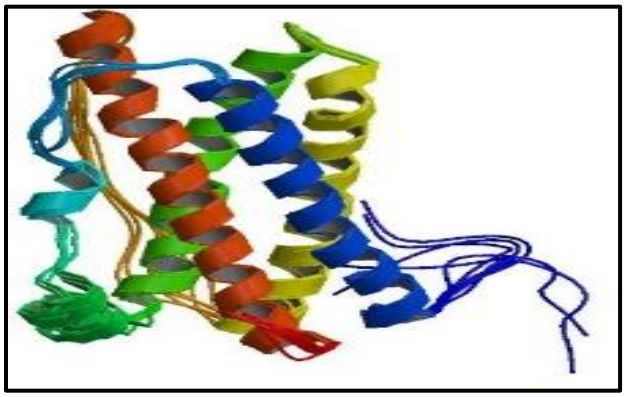

Fig 2.5: Structure of Prolactin

2.5. Cerebral Ischemic Models in Rodents

Different experimental models have been used for studying cerebral ischemia *in-vivo*, which can be broadly divided into focal, global and multifocal ischemia (Fig 2.6.) [103]. While global cerebral ischemia happens due to a reduction of blood flow throughout the brain or most of the brain regions, focal cerebral ischemia represents loss of blood flow in a specific and defined

area of brain [103]. Multi focal cerebral ischemia is caused when cerebral blood flow reduces in different patches of brain.

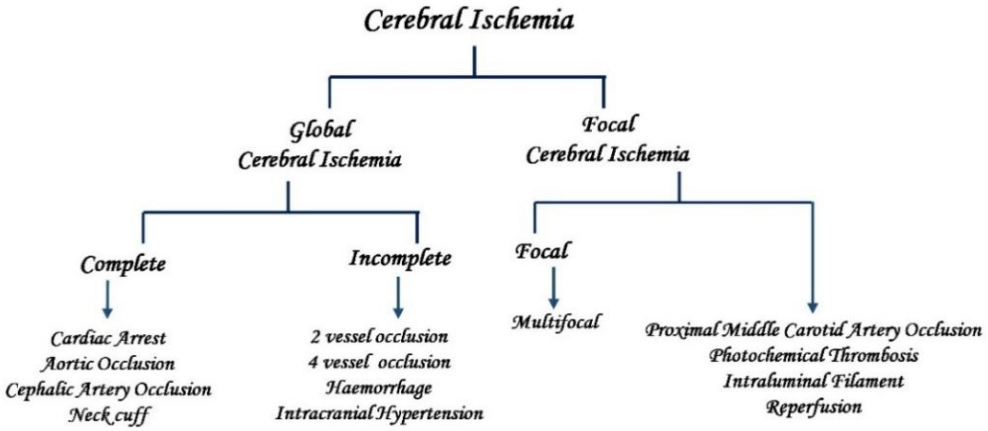

Fig. 2.6.: Types of experimental models in cerebral ischemia [103].

Induction of focal cerebral ischemia in rodent models involves obstructing the blood flow in brain via one major cerebral artery, mainly, middle cerebral artery (MCA) [104-106]. MCA occlusion (MCAO) lessens blood flow in striatal and cortical region depending on the site and duration of the occlusion [103]. While the early models of MCAO involved subtemporal craniotomy [107], the advancement of scientific studies have given birth to more sophisticated techniques. Recent techniques include electrocoagulation [103], photochemical occlusion of MCA [108] and insertion of intraluminal thread into MCA via common carotid artery [109]. Introduction of homologous blood clot in common carotid artery via a catheter through an external carotid artery is another easy and popular method of introducing focal cerebral ischemia, but inconsistent location of infarction development remains its major drawback [103]. The focal cerebral ischemia model is widely used since it is similar to thromboembolic stroke in human [104]. But this model is not sufficient for studying the effects of brain damage occurring due to a total loss of cerebral blood flow. This particular model is also associated with high mortality of experimental animals. Hence emerges the necessity of a reproducible, dependable and consistent animal model with low mortality rates to study biochemical and

physiological changes occurring as a result of global cerebral ischemia. Induction of global cerebral ischemia requires prevention of blood flow via the internal carotid arteries and vertebral arteries, which together forms the vascular structure termed as Circle of Willis (Fig. 2.7.) [111] and subsequent preservation of systemic blood flow reduces mortality rate. These four arteries form an anastomotic loop and retains perfusion in case of a proximal vascular occlusion hence blood flow via all these arteries needs to be obstructed to induce global cerebral ischemia [112]. The commonly used methods for inducing global cerebral ischemia are four vessel occlusion (4VO) and two vessel occlusion (2VO) methods [113-115]. To produce ischemic conditions in brain, the 4VO model requires an electrocauterization of the vertebral arteries via the alar foramina of the first cervical vertebra 24 hours prior to occlusion of the common carotid arteries [103]. Direct visualization of the vertebral arteries is difficult which limits the successful electrocauterization of these blood vessels [103]. This method also suffers from the involvement of surgical complexities, hindering successful implementation of the model. Hence, the 2VO model is more widely used in laboratory conditions to study ischemic pathophysiology and neuroprotection.

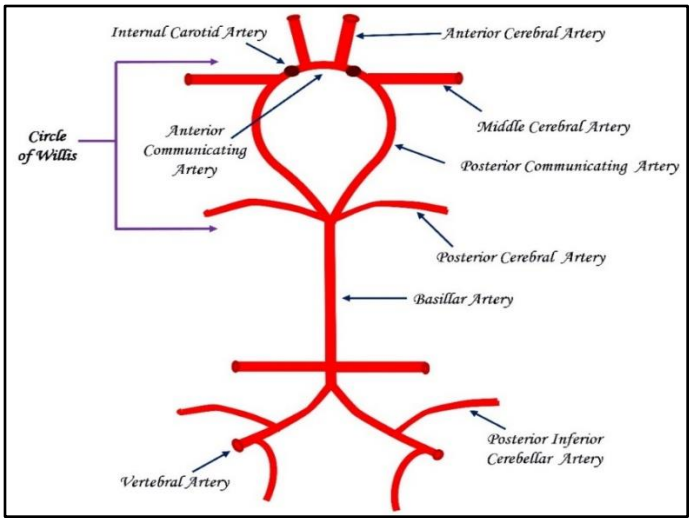

Fig. 2.7.: Representation of Circle of Willis

The 2VO model involves occlusion of the bilateral common carotid arteries (BCCA) (Fig. 2.8.) [103] and Iwasaki et al. demonstrated damage in brain tissue by BCCA occlusion (BCCAO) method [116]. BCCAO induced increase in superoxide dismutase activity, lipid peroxidation [117], decreased dendrite length and branching in CA1hippocampal region [118] has been observed in rat brain. Various studies have reportedly used 2VO model of BCCAO for studying ischemic pathophysiology and neuroprotection [119-121]. BCCAO has been also coupled with hypotension to obtain more significant results, but the model remains complex due to the absolute necessity of the animal to bleed [115, 122].

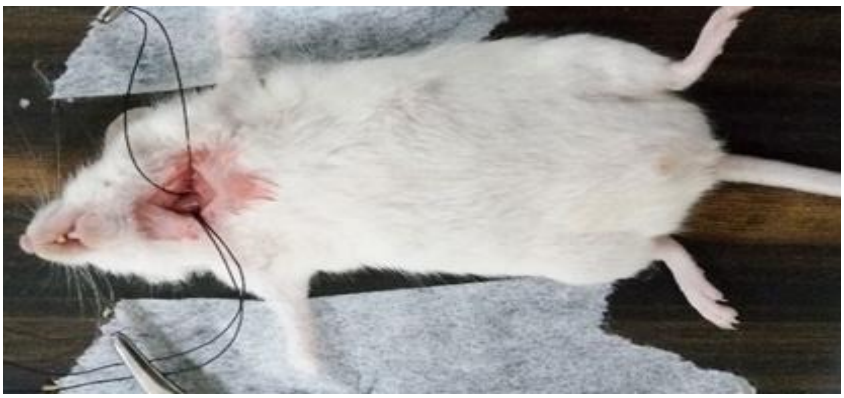

Fig. 2.8.: Induction of global cerebral ischemia by bilateral common carotid artery occlusion.

Though several rodent models have been developed to study cerebral ischemic pathologies and these models provide opportunities to explore mechanisms of neuropathology efficiently, none of them mimics or reproduces the exact nature of ischemic damage occurring in humans. This might be due to association of several other pathologies like diabetes, aging, elevated blood pressure with the pathophysiology of cerebral ischemia.

2.6. Molecular Docking Simulation in Drug Designing

Structure-based drug designing (SBDD) and virtual screening are potential new age tools for identifying potent lead compounds with pharmacological efficacy as inhibitors of different

enzymes, receptors and proteins involved as major mediators of profoundly significant pathophysiological pathways [123]. SBDD uses computational approaches to screen appropriate lead compounds as inhibitors by using 3 dimensional structures (3D) of the target protein or receptor [123]. 3D structures of target molecules can be easily retrieved from Protein data bank (PDB) which contains an extensive database of crystal/solution structures solved by X-ray crystallography and NMR techniques. The deposited structures are generally co-crystallized with various ligands (substrates or inhibitors), thus providing significant information regarding the catalytic/allosteric site of the enzyme/ protein and their binding characteristics [124]. These available data can be further exploited to gain an insight of the ligand-protein interactions and can be further used to successfully select potential inhibitors with active pharmacological properties. The interaction patterns between the ligands and the amino acid residues of the active/catalytic site of a target protein can be efficiently explored by using molecular docking techniques [125]. Molecular docking or virtual screening helps to predict the binding confirmation of the ligand along with predicting binding affinity and the orientation of the ligand in the binding site [126]. These computational approaches can also provide essential information regarding the formation of hydrogen bonds and hydrophobic interactions between the ligands and the amino acid residues of the catalytic/ active site of the target molecule. The primary step of molecular docking technique involves ligand conformation sampling into the docking site, which uses various sampling algorithms such as Genetic algorithms, Molecular dynamics, Monte Carlo, Incremental construction and Multiple Copy Simultaneous Search [127, 128]. The second step involves a scoring function, which uses force-field or empirical or knowledge-based functions to assess the binding affinity of different confirmations of the ligand with the target protein in order to rank the sampled confirmations [127]. AutoDock 4 is a software suite used to execute automated docking studies

and uses a semi-empirical free energy force field to evaluate the ligand confirmations obtained during docking studies [129]. AutoDock 4 is endowed with proficient search performances since it is equipped with the traditional genetic algorithm, a combination of genetic algorithm and local optimization inheritance, thus yielding the Lamarckian genetic algorithm and the simulated annealing [129]. The software allows the user to select any of these three algorithms, although Lamarckian genetic algorithm is reported to provide most efficient and reproducible results [130].

References

1. Liu Z, Liu Y, Zhou H, Fu X, Hu G. Epoxyeicosatrienoic acid ameliorates cerebral ischemia-reperfusion injury by inhibiting inflammatory factors and pannexin-1. Molecular medicine reports. 2017 Aug 1;16(2):2179-84. doi:10.3892/mmr.2017.6831.
2. Lo EH, Dalkara T, Moskowitz MA. Neurological diseases: Mechanisms, challenges and opportunities in stroke. Nature reviews neuroscience. 2003 May;4(5):399. doi: 10.1038/nrn1106.
3. Mehta SL, Manhas N, Raghubir R. Molecular targets in cerebral ischemia for developing novel therapeutics. Brain research reviews. 2007 Apr 1;54(1):34-66. doi: 10.1016/j.brainresrev.2006.11.003.
4. Endres M, Wang ZQ, Namura S, Waeber C, Moskowitz MA. Ischemic brain injury is mediated by the activation of poly (ADP-ribose) polymerase. Journal of Cerebral Blood Flow & Metabolism. 1997 Nov;17(11):1143-51. doi: 10.1097/00004647-199711000-00002.
5. Andrabi SA, Kim NS, Yu SW, Wang H, Koh DW, Sasaki M, Klaus JA, Otsuka T, Zhang Z, Koehler RC, Hurn PD. Poly (ADP-ribose)(PAR) polymer is a death signal. Proceedings of the National Academy of Sciences. 2006 Nov 28;103(48):18308-13. doi: 10.1073/pnas.0606526103.
6. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nature chemical biology. 2005 May 29;1(2):112. doi: 10.1038/nchembio711.
7. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J. Fas triggers an alternative, caspase-8–independent cell death pathway using the kinase RIP as effector molecule. Nature immunology. 2000 Dec;1(6):489. doi: 10.1038/82732.
8. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, Abbott D, Cuny GD, Yuan C, Wagner G, Hedrick SM. Identification of RIP1 kinase as a specific cellular target of necrostatins. Nature chemical biology. 2008 May;4(5):313. doi: 10.1038/nchembio.83.
9. Jang KH, Jang T, Son E, Choi S, Kim E. Kinase-independent role of nuclear RIPK1 in regulating parthanatos through physical interaction with PARP1 upon oxidative stress. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 1865(1). 2018. 132-41. doi: 10.1016/j.bbamcr.2017.10.004.
10. Thompson JE. The evolution of surgery for the treatment and prevention of stroke: The Willis Lecture. Stroke. 1996 Aug;27(8):1427-34. doi: 10.1161/01.STR.27.8.1427.
11. Kelly MA. Stroke: a modern history. American journal of therapeutics. 2011 Jan 1;18(1):51-6. doi:10.1097/MJT.0b013e3181e13a0b.
12. Hasan TF, Rabinstein AA, Middlebrooks EH, Haranhalli N, Silliman SL, Meschia JF, Tawk RG. Diagnosis and management of acute ischemic stroke. InMayo Clinic

Proceedings 2018 Apr 1 (Vol. 93, No. 4, pp. 523-538). Elsevier. doi: 10.1016/j.mayocp.2018.02.013.

13. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, Schonewille WJ, Vos JA, Nederkoorn PJ, Wermer MJ, van Walderveen MA. A randomized trial of intraarterial treatment for acute ischemic stroke. New England Journal of Medicine. 2015 Jan 1;372(1):11-20. doi: 10.1056/NEJMoa1411587.
14. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, Albers GW, Cognard C, Cohen DJ, Hacke W, Jansen O. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. New England Journal of Medicine. 2015 Jun 11;372(24):2285-95. doi: 10.1056/NEJMoa1415061.
15. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, Yan B, Dowling RJ, Parsons MW, Oxley TJ, Wu TY. Endovascular therapy for ischemic stroke with perfusion-imaging selection. New England Journal of Medicine. 2015 Mar 12;372(11):1009-18. doi: 10.1056/NEJMoa1414792.
16. Sarraj A, Hassan A, Savitz SI, Grotta JC, Cai C, Parsha KN, Farrell CM, Imam B, Sitton CW, Reddy ST, Kamal H. Endovascular Thrombectomy for Mild Strokes: How Low Should We Go? A Multicenter Cohort Study. Stroke. 2018 Oct;49(10):2398-405. doi: 10.1161/STROKEAHA.118.022114.
17. Rabinstein AA. Treatment of acute ischemic stroke. CONTINUUM: Lifelong Learning in Neurology. 2017 Feb 1;23(1, Cerebrovascular Disease):62-81. doi: 10.1212/CON.0000000000000420.
18. Hass WK, Easton JD, Adams Jr HP, Pryse-Phillips W, Molony BA, Anderson S, Kamm B, Ticlopidine Aspirin Stroke Study Group. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. New England Journal of Medicine. 1989 Aug 24;321(8):501-7. doi: 10.1056/NEJM198908243210804.
19. Sandercock PA, Counsell C, Kane EJ. Anticoagulants for acute ischaemic stroke. Cochrane Database of Systematic Reviews. 2015(3). doi:10.1002/14651858.CD000024.pub4.
20. Moroni F, Chiarugi A. Post- ischemic brain damage: targeting PARP- 1 within the ischemic neurovascular units as a realistic avenue to stroke treatment. The FEBS journal. 2009 Jan 1;276(1):36-45. doi:10.1111/j.1742-4658.2008.06768.x.
21. Ginsberg MD. Neuroprotection for ischemic stroke: past, present and future. Neuropharmacology. 2008 Sep 1;55(3):363-89. doi:10.1016/j.neuropharm.2007.12.007.
22. Sutherland BA, Minnerup J, Balami JS, Arba F, Buchan AM, Kleinschnitz C. Neuroprotection for ischaemic stroke: translation from the bench to the bedside. International Journal of Stroke. 2012 Jul;7(5):407-18. doi: 10.1111/j.1747-4949.2012.00770.x.

23. Xiong XY, Liu L, Yang QW. Refocusing neuroprotection in cerebral reperfusion era: new challenges and strategies. Frontiers in neurology. 2018;9. doi: 10.3389/fneur.2018.00249.
24. Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: identifying novel targets for neuroprotection. Progress in neurobiology. 2014 Apr 1;115:157-88. doi: 10.1016/j.pneurobio.2013.11.006.
25. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. Annals of neurology. 2006 Mar;59(3):467-77. doi: 10.1002/ana.20741.
26. Zhang JH, Badaut J, Tang J, Obenaus A, Hartman R, Pearce WJ. The vascular neural network—a new paradigm in stroke pathophysiology. Nature Reviews Neurology. 2012 Dec;8(12):711. doi: 10.1038/nrneurol.2012.210.
27. Szabó C, Dawson VL. Role of poly (ADP-ribose) synthetase in inflammation and ischaemia–reperfusion. Trends in Pharmacological Sciences. 1998 Jul 1;19(7):287-98. doi: 10.1016/S0165-6147(98)01193-6.
28. Homburg S, Visochek L, Moran N, Dantzer F, Priel E, Asculai E, Schwartz D, Rotter V, Dekel N, Cohen-Armon M. A fast signal–induced activation of poly (ADP-ribose) polymerase: a novel downstream target of phospholipase C. The Journal of cell biology. 2000 Jul 24;150(2):293-308. doi: 10.1083/jcb.150.2.293.
29. Szabo C, Pacher P, Swanson RA. Novel modulators of poly (ADP-ribose) polymerase. Trends in pharmacological sciences. 2006 Dec 1;27(12):626-30. doi: 10.1016/j.tips.2006.10.003.
30. Alano CC, Swanson RA. Players in the PARP-1 cell-death pathway: JNK1 joins the cast. Trends in biochemical sciences. 2006 Jun 1;31(6):309-11. doi: 10.1016/j.tibs.2006.04.006.
31. Haddad M, Rhinn H, Bloquel C, Coqueran B, Szabo C, Plotkine M, Scherman D, Margaill I. Anti- inflammatory effects of PJ34, a poly (ADP- ribose) polymerase inhibitor, in transient focal cerebral ischemia in mice. British journal of pharmacology. 2006 Sep 1;149(1):23-30. doi: 10.1038/sj.bjp.0706837.
32. Lo EH, Bosque-Hamilton P, Meng W. Inhibition of PARP: reduction of ischemic injury and attenuation of NMDA-induced neurotransmitter dysregulation. Stroke. 1998;29(4):830-6. doi: 10.1161/01.STR.29.4.830.
33. Zhang S, Tang MB, Luo HY, Shi CH, Xu YM. Necroptosis in neurodegenerative diseases: a potential therapeutic target. Cell death & disease. 2017 Jun;8(6):e2905. doi:10.1038/cddis.2017.286.
34. Yang SH, Lee DK, Shin J, Lee S, Baek S, Kim J, Jung H, Hah JM, Kim Y. Nec- 1 alleviates cognitive impairment with reduction of Aβ and tau abnormalities in APP/PS1 mice. EMBO molecular medicine. 2017 Jan 1;9(1):61-77. doi: 10.15252/emmm.201606566.
35. Qinli Z, Meiqing L, Xia J, Li X, Weili G, Xiuliang J, Junwei J, Hailan Y, Ce Z, Qiao N. Necrostatin-1 inhibits the degeneration of neural cells induced by aluminum

exposure. Restorative neurology and neuroscience. 2013 Jan 1;31(5):543-55. doi: 10.3233/RNN-120304.

36. Wu JR, Wang J, Zhou SK, Yang L, Yin JL, Cao JP, Cheng YB. Necrostatin-1 protection of dopaminergic neurons. Neural regeneration research. 2015 Jul;10(7):1120. doi: 10.4103/1673-5374.160108.
37. Zhu S, Zhang Y, Bai G, Li H. Necrostatin-1 ameliorates symptoms in R6/2 transgenic mouse model of Huntington's disease. Cell death & disease. 2011 Jan 13;2(1):e115. doi: doi.org/10.1038/cddis.2010.94.
38. Ito Y, Ofengeim D, Najafov A, Das S, Saberi S, Li Y, Hitomi J, Zhu H, Chen H, Mayo L, Geng J. RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. Science. 2016 Aug 5;353(6299):603-8. doi: 10.1126/science.aaf6803.
39. Re DB, Le Verche V, Yu C, Amoroso MW, Politi KA, Phani S, Ikiz B, Hoffmann L, Koolen M, Nagata T, Papadimitriou D. Necroptosis drives motor neuron death in models of both sporadic and familial ALS. Neuron. 2014 Mar 5;81(5):1001-8. doi: 10.1016/j.neuron.2014.01.011.
40. Hughes MA, Powley IR, Jukes-Jones R, Horn S, Feoktistova M, Fairall L, Schwabe JW, Leverkus M, Cain K, MacFarlane M. Co-operative and hierarchical binding of c-FLIP and caspase-8: a unified model defines how c-FLIP isoforms differentially control cell fate. Molecular cell. 2016 Mar 17;61(6):834-49. doi: 10.1016/j.molcel.2016.02.023.
41. Jagtap PG, Degterev A, Choi S, Keys H, Yuan J, Cuny GD. Structure− activity relationship study of tricyclic necroptosis inhibitors. Journal of medicinal chemistry. 2007 Apr 19;50(8):1886-95. doi: 10.1021/jm061016o.
42. Bigford GE, Del Rossi G. Supplemental substances derived from foods as adjunctive therapeutic agents for treatment of neurodegenerative diseases and disorders. Advances in Nutrition. 2014 Jul 7;5(4):394-403. doi: 10.3945/an.113.005264.
43. Bernier M, Wahl D, Ali A, Allard J, Faulkner S, Wnorowski A, Sanghvi M, Moaddel R, Alfaras I, Mattison JA, Tarantini S. Resveratrol supplementation confers neuroprotection in cortical brain tissue of nonhuman primates fed a high-fat/sucrose diet. Aging (Albany NY). 2016 May;8(5):899. doi: 10.18632/aging.100942.
44. Pasinetti GM, Wang J, Ho L, Zhao W, Dubner L. Roles of resveratrol and other grape-derived polyphenols in Alzheimer's disease prevention and treatment. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2015 Jun 1;1852(6):1202-8. doi: 10.1016/j.bbadis.2014.10.006.
45. Han YS, Zheng WH, Bastianetto S, Chabot JG, Quirion R. Neuroprotective effects of resveratrol against β- amyloid- induced neurotoxicity in rat hippocampal neurons: involvement of protein kinase C. British journal of pharmacology. 2004 Mar;141(6):997-1005. doi: 10.1038/sj.bjp.0705688.
46. Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, Cole GM. Phenolic anti-inflammatory antioxidant reversal of Aβ-induced cognitive deficits and

neuropathology. Neurobiology of aging. 2001 Nov 1;22(6):993-1005. doi: 10.1016/S0197-4580(01)00300-1.
47. Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. Journal of Neuroscience. 2001 Nov 1;21(21):8370-7. doi: 10.1523/JNEUROSCI.21-21-08370.2001.
48. Wang MS, Boddapati S, Emadi S, Sierks MR. Curcumin reduces α-synuclein induced cytotoxicity in Parkinson's disease cell model. BMC neuroscience. 2010 Dec;11(1):57. doi: 10.1186/1471-2202-11-57.
49. Jones QR, Warford J, Rupasinghe HV, Robertson GS. Target-based selection of flavonoids for neurodegenerative disorders. Trends in pharmacological sciences. 2012 Nov 1;33(11):602-10. doi: 10.1016/j.tips.2012.08.002.
50. Bhullar KS, Rupasinghe HP. Polyphenols: multipotent therapeutic agents in neurodegenerative diseases. Oxidative medicine and cellular longevity. 2013 Jun 6;2013. doi: 10.1155/2013/891748.
51. Celik S, Erdogan S. Caffeic acid phenethyl ester (CAPE) protects brain against oxidative stress and inflammation induced by diabetes in rats. Molecular and cellular biochemistry. 2008 May 1;312(1-2):39-46. doi: 10.1007/s11010-008-9719-3.
52. Noelker C, Bacher M, Gocke P, Wei X, Klockgether T, Du Y, Dodel R. The flavanoide caffeic acid phenethyl ester blocks 6-hydroxydopamine-induced neurotoxicity. Neuroscience letters. 2005 Jul 22;383(1-2):39-43. doi: 10.1016/j.neulet.2005.04.023.
53. Scapagnini G, Foresti R, Calabrese V, Stella AG, Green CJ, Motterlini R. Caffeic acid phenethyl ester and curcumin: a novel class of heme oxygenase-1 inducers. Molecular pharmacology. 2002 Mar 1;61(3):554-61. doi:10.1124/mol.61.3.554.
54. Ahmed T, Raza SH, Maryam A, Setzer WN, Braidy N, Nabavi SF, de Oliveira MR, Nabavi SM. Ginsenoside Rb1 as a neuroprotective agent: a review. Brain research bulletin. 2016 Jul 1;125:30-43. doi: 10.1016/j.brainresbull.2016.04.002.
55. Cai M, Yang EJ. Ginsenoside Re attenuates neuroinflammation in a symptomatic ALS animal model. The American journal of Chinese medicine. 2016 Apr 1;44(02):401-13. doi: 10.1142/S0192415X16500233.
56. Zhang X, Wang Y, Ma C, Yan Y, Yang Y, Wang X, Rausch WD. Ginsenoside Rd and ginsenoside Re offer neuroprotection in a novel model of Parkinson's disease. American journal of neurodegenerative disease. 2016;5(1):52. PMID: 27073742.
57. Qian-hang S, Yu-he Y, Nai-hong C. Ginsenoside Rg1 attenuates motor impairment and neuroinflammation in the MPTP-probenecid-induced parkinsonism mouse model. Chinese Journal of Pharmacology and Toxicology. 2017 Jan 1;31(10):999-1000. doi: 10.3867/j.issn.1000-3002.2017.10.080.
58. Hsu YY, Tseng YT, Lo YC. Berberine, a natural antidiabetes drug, attenuates glucose neurotoxicity and promotes Nrf2-related neurite outgrowth. Toxicology and applied pharmacology. 2013 Nov 1;272(3):787-96. doi: 10.1016/j.taap.2013.08.008.

59. Zhang Q, Qian Z, Pan L, Li H, Zhu H. Hypoxia-inducible factor 1 mediates the anti-apoptosis of berberine in neurons during hypoxia/ischemia. Acta Physiologica Hungarica. 2012 Sep 1;99(3):311-23. doi: 10.1556/APhysiol.99.2012.3.8.
60. Dhuley JN. Effect of ashwagandha on lipid peroxidation in stress-induced animals. Journal of ethnopharmacology. 1998 Mar 1;60(2):173-8. doi:10.1016/S0378-8741(97)00151-7.
61. Baitharu I, Jain V, Deep SN, Shroff S, Sahu JK, Naik PK, Ilavazhagan G. Withanolide A prevents neurodegeneration by modulating hippocampal glutathione biosynthesis during hypoxia. PloS one. 9(10). 2014. e105311. doi: 10.1371/journal.pone.0105311.
62. Kurapati KR, Atluri VS, Samikkannu T, Nair MP. Ashwagandha (Withania somnifera) reverses β-amyloid1-42 induced toxicity in human neuronal cells: implications in HIV-associated neurocognitive disorders (HAND). PLoS One. 2013 Oct 16;8(10):e77624. doi: 10.1371/journal.pone.0077624.
63. Kuboyama T, Tohda C, Komatsu K. Neuritic regeneration and synaptic reconstruction induced by withanolide A. British journal of pharmacology. 144(7). 2005. 961-71. doi: 10.1038/sj.bjp.0706122.
64. Kuboyama T, Tohda C, Zhao J, Nakamura N, Hattori M, Komatsu K. Axon-or dendrite-predominant outgrowth induced by constituents from Ashwagandha. Neuroreport. 13(14). 2002. 1715-20. PMID: 12395110.
65. Akhoon BA, Pandey S, Tiwari S, Pandey R. Withanolide A offers neuroprotection, ameliorates stress resistance and prolongs the life expectancy of Caenorhabditis elegans. Experimental gerontology. 78. 2016. 47-56. doi:10.1016/j.exger.2016.03.004.
66. Baitharu I, Jain V, Deep SN, Shroff S, Sahu JK, Naik PK, Ilavazhagan G. Withanolide A prevents neurodegeneration by modulating hippocampal glutathione biosynthesis during hypoxia. PloS one. 2014 Oct 13;9(10):e105311. doi: 10.1371/journal.pone.0105311.
67. Saiyed M, Riker WK. Cholinergic and anticholinergic drug effects on survival during hypoxia: significant gender differences. Journal of Pharmacology and Experimental Therapeutics. 1993 Mar 1;264(3):1146-53. PubMed: 8450456.
68. Cadet JL, Ladenheim B, Baum I, Carlson E, Epstein C. CuZn-superoxide dismutase (CuZnSOD) transgenic mice show resistance to the lethal effects of methylenedioxyamphetamine (MDA) and of methylenedioxymethamphetamine (MDMA). Brain research. 1994 Aug 29;655(1-2):259-62. doi: 10.1016/0006-8993(94)91624-1.
69. Roof RL, Duvdevani R, Stein DG. Gender influences outcome of brain injury: progesterone plays a protective role. Brain research. 1993 Apr 2;607(1-2):333-6. doi: 10.1016/0006-8993(93)91526-X.
70. Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD. Gender-linked brain injury in experimental stroke. Stroke. 1998 Jan 1;29:159-65. doi: 10.1161/01.STR.29.1.159.

71. Vannucci SJ, Willing LB, Goto S, Alkayed NJ, Brucklacher RM, Wood TL, Towfighi J, Hurn PD, Simpson IA. Experimental stroke in the female diabetic, db/db, mouse. Journal of Cerebral Blood Flow & Metabolism. 2001 Jan;21(1):52-60. doi: 10.1097/00004647-200101000-00007.
72. Coelingh Bennink HJ, Holinka CF, Diczfalusy E. Estetrol review: profile and potential clinical applications. Climacteric. 2008 Jan 1;11(sup1):47-58. doi:10.1080/13697130802073425.
73. De Butte-Smith M, Gulinello M, Zukin RS, Etgen AM. Chronic estradiol treatment increases CA1 cell survival but does not improve visual or spatial recognition memory after global ischemia in middle-aged female rats. Hormones and behavior. 2009 Mar 1;55(3):442-53. doi: 10.1016/j.yhbeh.2008.11.011.
74. Miller NR, Jover T, Cohen HW, Zukin RS, Etgen AM. Estrogen can act via estrogen receptor α and β to protect hippocampal neurons against global ischemia-induced cell death. Endocrinology. 2005 Jul 1;146(7):3070-9. doi: 10.1210/en.2004-1515.
75. Jover-Mengual T, Zukin RS, Etgen AM. MAPK signaling is critical to estradiol protection of CA1 neurons in global ischemia. Endocrinology. 2007 Mar 1;148(3):1131-43. doi: 10.1210/en.2006-1137.
76. Sribnick EA, Ray SK, Nowak MW, Li L, Banik NL. 17β- estradiol attenuates glutamate- induced apoptosis and preserves electrophysiologic function in primary cortical neurons. Journal of neuroscience research. 2004 Jun 1;76(5):688-96. doi: 10.1002/jnr.20124.
77. Bryant DN, Sheldahl LC, Marriott LK, Shapiro RA, Dorsa DM. Multiple pathways transmit neuroprotective effects of gonadal steroids. Endocrine. 2006 Apr 1;29(2):199-207. doi: 10.1385/ENDO:29:2:199.
78. Singer CA, Rogers KL, Dorsa DM. Modulation of Bcl-2 expression: a potential component of estrogen protection in NT2 neurons. Neuroreport. 1998 Aug 3;9(11):2565-8. PMID: 9721933.
79. Stirone C, Boroujerdi A, Duckles SP, Krause DN. Estrogen receptor activation of phosphoinositide-3 kinase, akt, and nitric oxide signaling in cerebral blood vessels: rapid and long-term effects. Molecular pharmacology. 2005 Jan 1;67(1):105-13. doi: 10.1124/mol.104.004465.
80. Vegeto E, Benedusi V, Maggi A. Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. Frontiers in neuroendocrinology. 2008 Oct 1;29(4):507-19. doi: 10.1016/j.yfrne.2008.04.001.
81. Lewis DK, Johnson AB, Stohlgren S, Harms A, Sohrabji F. Effects of estrogen receptor agonists on regulation of the inflammatory response in astrocytes from young adult and middle-aged female rats. Journal of neuroimmunology. 2008 Mar 1;195(1-2):47-59. doi: 10.1016/j.jneuroim.2008.01.006.
82. Draca S. Estriol and progesterone: a new role for sex hormones. International journal of biomedical science: IJBS. 2006 Dec;2(4):305. PMID: 23674997.

83. Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TJ, Voskuhl RR. Treatment of multiple sclerosis with the pregnancy hormone estriol. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 2002 Oct;52(4):421-8. doi: 10.1002/ana.10301.
84. Soldan SS, Retuerto AI, Sicotte NL, Voskuhl RR. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. The Journal of Immunology. 2003 Dec 1;171(11):6267-74. PMID: 14634144.
85. Tskitishvili E, Nisolle M, Munaut C, Pequeux C, Gerard C, Noel A, Foidart JM. Estetrol attenuates neonatal hypoxic–ischemic brain injury. Experimental neurology. 2014 Nov 1;261:298-307. doi: 10.1016/j.expneurol.2014.07.015.
86. Liu M, Kelley MH, Herson PS, Hurn PD. Neuroprotection of sex steroids. Minerva endocrinologica. 2010 Jun;35(2):127. PMID: 20595940.
87. Gibson CL, Constantin D, Prior MJ, Bath PM, Murphy SP. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. Experimental neurology. 2005 Jun 1;193(2):522-30. doi: 10.1016/j.expneurol.2005.01.009.
88. Aggarwal R, Medhi B, Pathak A, Dhawan V, Chakrabarti A. Neuroprotective effect of progesterone on acute phase changes induced by partial global cerebral ischaemia in mice. Journal of Pharmacy and Pharmacology. 2008 Jun;60(6):731-7. doi: 10.1211/jpp.60.6.0008.
89. Wang J, Jiang C, Liu C, Li X, Chen N, Hao Y. Neuroprotective effects of progesterone following stroke in aged rats. Behavioural brain research. 2010 May 1;209(1):119-22. doi: 10.1016/j.bbr.2010.01.026.
90. Jiang C, Wang J, Li X, Liu C, Chen N, Hao Y. Progesterone exerts neuroprotective effects by inhibiting inflammatory response after stroke. Inflammation research. 2009 Sep 1;58(9):619-24. doi: 10.1007/s00011-009-0032-8.
91. Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. Endocrine reviews. 1996 Dec 1;17(6):639-69. doi: 10.1210/edrv-17-6-639.
92. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiological reviews. 2000 Jan 10;80(4):1523-631. doi: 10.1152/physrev.2000.80.4.1523.
93. De Vito WJ, Stone S, Shamgochian M. Prolactin induced expression of glial fibrillary acidic protein and tumor necrosis factor-alpha at a wound site in the rat brain. Molecular and cellular endocrinology. 1995 Feb 27;108(1-2):125-30. doi: 10.1016/0303-7207(94)03465-6.
94. Torner L, Neumann ID. The brain prolactin system: involvement in stress response adaptations in lactation. Stress. 2002 Jan 1;5(4):249-57. doi: 10.1080/1025389021000048638.
95. Walker TL, Vukovic J, Koudijs MM, Blackmore DG, Mackay EW, Sykes AM, Overall RW, Hamlin AS, Bartlett PF. Prolactin stimulates precursor cells in the adult

mouse hippocampus. PLoS One. 2012 Sep 4;7(9):e44371. doi: 10.1371/journal.pone.0044371.

96. Gregg C. Pregnancy, prolactin and white matter regeneration. Journal of the neurological sciences. 2009 Oct 15;285(1-2):22-7. doi: 10.1016/j.jns.2009.06.040.
97. Gregg C, Shikar V, Larsen P, Mak G, Chojnacki A, Yong VW, Weiss S. White matter plasticity and enhanced remyelination in the maternal CNS. Journal of Neuroscience. 2007 Feb 21;27(8):1812-23. doi: 10.1523/JNEUROSCI.4441-06.2007.
98. Cabrera V, Cantú D, Ramos E, Vanoye-Carlo A, Cerbón M, Morales T. Lactation is a natural model of hippocampus neuroprotection against excitotoxicity. Neuroscience letters. 2009 Sep 11;461(2):136-9. doi: 10.1016/j.neulet.2009.06.017.
99. Vanoye-Carlo A, Morales T, Ramos E, Mendoza-Rodríguez A, Cerbón M. Neuroprotective effects of lactation against kainic acid treatment in the dorsal hippocampus of the rat. Hormones and behavior. 2008 Jan 1;53(1):112-23. doi: 10.1016/j.yhbeh.2007.09.004.
100. Vergara-Castañeda E, Grattan DR, Pasantes-Morales H, Pérez-Domínguez M, Cabrera-Reyes EA, Morales T, Cerbón M. Prolactin mediates neuroprotection against excitotoxicity in primary cell cultures of hippocampal neurons via its receptor. Brain research. 2016 Apr 1;1636:193-9. doi: 10.1016/j.brainres.2016.02.011.
101. Rivero-Segura, N.A., Flores-Soto, E., de la Cadena, S.G., Coronado-Mares, I., Gomez-Verjan, J.C., Ferreira, D.G., Cabrera-Reyes, E.A., Lopes, L.V., Massieu, L. and Cerbón, M., 2017. Prolactin-induced neuroprotection against glutamate excitotoxicity is mediated by the reduction of [Ca2+] i overload and NF-κB activation. PloS one, 12(5),p.e0176910. doi:10.1371/journal.pone.0176910.
102. Traystman RJ. Animal models of focal and global cerebral ischemia. ILAR journal. 2003 Jan 1;44(2):85-95. doi: 10.1093/ilar.44.2.85.
103. Garcia JH. Experimental ischemic stroke: a review. Stroke. 1984 Jan;15(1):5-14. PMID: 6364464.
104. Hossmann KA. Animal models of cerebral ischemia. 1. Review of literature. Cerebrovascular Diseases. 1991;1(Suppl. 1):2-15. doi: 10.1159/000108875.
105. Ponten U, Ratcheson RA, Salford LG, Siesjö BK. Optimal freezing conditions for cerebral metabolites in rats. Journal of neurochemistry. 1973 Nov;21(5):1127-38. doi: 10.1111/j.1471-4159.1973.tb07567.x.
106. Tamura A. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab. 1981;1:53-60. doi: 10.1038/jcbfm.1981.6.
107. Watson BD. Animal models of photochemically induced brain ischemia and stroke. Cerebrovascular disease. Pathophysiology, diagnosis, and management. 1998;1:52-74.
108. Koizumi JY. Experimental studies of ischemic brain edema. 1. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn J stroke. 1986;8:1-8. doi: 10.3995/jstroke.8.1.

109. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. stroke. 1989 Jan;20(1):84-91. PMID: 2643202.
110. Hendrikse J, van Raamt AF, van der Graaf Y, Mali WP, van der Grond J. Distribution of cerebral blood flow in the circle of Willis. Radiology. 2005 Apr;235(1):184-9. doi: 10.1148/radiol.2351031799.
111. Sanderson TH, Wider JM. 2-vessel occlusion/hypotension: a rat model of global brain ischemia. JoVE (Journal of Visualized Experiments). 2013 Jun 22(76):e50173. doi: 10.3791/50173.
112. Pulsinelli WA, Buchan AM. The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke. 1988 Jul 1;19(7):913-4. PMID: 3291205.
113. Pulsinelli WA, Duffy TE. Regional energy balance in rat brain after transient forebrain ischemia. Journal of neurochemistry. 1983 May;40(5):1500-3. doi: 10.1111/j.1471-4159.1983.tb13599.x.
114. Eklöf B, Siesjö BK. The effect of bilateral carotid artery ligation upon acid- base parameters and substrate levels in the rat brain. Acta Physiologica Scandinavica. 1972 Dec;86(4):528-38. doi: 10.1111/j.1748-1716.1972.tb05354.x.
115. Iwasaki Y, Ito S, Suzuki M, Nagahori T, Yamamoto T, Konno H. Forebrain ischemia induced by temporary bilateral common carotid occlusion in normotensive rats. Journal of the neurological sciences. 1989 Apr 1;90(2):155-65. doi: 10.1016/0022-510X(89)90098-1.
116. Yanpallewar SU, Hota D, Rai S, Kumar M, Acharya SB. Nimodipine attenuates biochemical, behavioral and histopathological alterations induced by acute transient and long-term bilateral common carotid occlusion in rats. Pharmacological research. 2004 Feb 1;49(2):143-50. doi: 10.1016/j.phrs.2003.08.005.
117. Jia H, Zhang XM, Zhang BA, Liu Y, Li JM. Dendritic morphology of neurons in medial prefrontal cortex and hippocampus in 2VO rats. Neurological Sciences. 2012 Oct 1;33(5):1063-70. doi: 10.1007/s10072-011-0898-4.
118. Pourheydar B, Soleimani Asl S, Azimzadeh M, Rezaei Moghadam A, Marzban A, Mehdizadeh M. Neuroprotective effects of bone marrow mesenchymal stem cells on bilateral common carotid arteries occlusion model of cerebral ischemia in rat. Behavioural neurology. 2016;2016. doi: 10.1155/2016/2964712.
119. Liu Z, Chen X, Gao Y, Sun S, Yang L, Yang Q, Bai F, Xiong L, Wang Q. Involvement of GluR2 up-regulation in neuroprotection by electroacupuncture pretreatment via cannabinoid CB1 receptor in mice. Scientific reports. 2015 Apr 1;5:9490. doi: 10.1038/srep09490.
120. Pujari RR, Vyawahare NS, Kagathara VG. Evaluation of antioxidant and neuroprotective effect of date palm (Phoenix dactylifera L.) against bilateral common carotid artery occlusion in rats. PMID: 21870431.

121. Smith ML, Auer RN, Siesjö BK. The density and distribution of ischemic brain injury in the rat following 2–10 min of forebrain ischemia. Acta neuropathologica. 1984 Dec 1;64(4):319-32. PMID: 6507048.
122. Lionta E, Spyrou G, K Vassilatis D, Cournia Z. Structure-based virtual screening for drug discovery: principles, applications and recent advances. Current topics in medicinal chemistry. 2014 Aug 1;14(16):1923-38. doi: 10.2174/1568026614666140929124445.
123. Müller I. Guidelines for the successful generation of protein–ligand complex crystals. Acta Crystallographica Section D: Structural Biology. 2017 Feb 1;73(2):79-92. doi: 10.1107/S2059798316020271.
124. Taylor RD, Jewsbury PJ, Essex JW. A review of protein-small molecule docking methods. Journal of computer-aided molecular design. 2002 Mar 1;16(3):151-66. doi: 10.1023/A:1020155510718.
125. Lybrand TP. Ligand—protein docking and rational drug design. Current opinion in structural biology. 1995 Apr 1;5(2):224-8. PMID: 7648325.
126. Morris GM, Lim-Wilby M. Molecular docking. In Molecular modeling of proteins 2008 (pp. 365-382). Humana Press. doi:10.1007/978-1-59745-177-2_19.
127. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Current computer-aided drug design. 2011 Jun 1;7(2):146-57. doi:10.1016/j.biotechadv.2011.08.021.
128. Hill AD, Reilly PJ. Scoring functions for AutoDock. InGlycoinformatics 2015 (pp. 467-474). Humana Press, New York, NY. doi:10.1007/978-1-4939-2343-4_27.
129. Casadevall A, Bik EM, Fang FC, Kullas A, Davis RJ. Analysis and Correction of Inappropriate Image Duplication: The Molecular and Cellular Biology Experience. BioRxiv. 2018 Jan 1:354621. doi:10.1101/354621.