Review Article

Klotho Protein in Neuronal Disorders: Exploring Molecular Mechanisms and Therapeutic Interventions

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ABSTRACT

Neurological disorders encompass a broad spectrum of conditions affecting the central and peripheral nervous systems, with etiologies that include genetic predisposition, infections, trauma, and environmental factors. These disorders, which include neurodegenerative diseases, cerebrovascular disorders, neuropsychiatric conditions, neurodevelopmental disorders, neurotraumatic injuries, movement disorders, multiple sclerosis, migraines, and peripheral neuropathies, present a substantial global healthcare challenge due to their high prevalence and significant impact on quality of life. The complexity of the nervous system, combined with the multifactorial nature of these disorders, presents significant obstacles to therapeutic intervention, underscoring the need for a more refined understanding of their underlying molecular mechanisms. The Klotho protein has garnered attention for its role in attenuating the aging process, with elevated expression in the brain shown to ameliorate the pathological features associated with neurological disorders through the modulation of key molecular signaling pathways. This review aims to provide a comprehensive analysis of the molecular mechanisms by which Klotho exerts its neuroprotective effects, while also exploring current and emerging therapeutic strategies targeting Klotho as a potential avenue for the development of novel treatments for neurological disorders.

Keywords: Neuronal diseases, klotho, genomics, molecular signaling, therapeutics, SDGs:SDG 3: Good Health and Well-being; SDG 10: Reduced Inequalities.

INTRODUCTION

Neurological disorders are a diverse group of diseases that are marked by the impairment in the normal functioning of brain or spinal cord. These disorders are sub classified into neuropsychiatric (such as dyskinesia, epilepsy, depression, anxiety etc.) neurotraumatic (such as brain injury, spinal cord injury, stroke, etc.), and neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, Huntington's disease, etc.[1] A recent data of WHO states that one third of global population is affected by the neurological disorders [2]. The Global Burden of Disease, injuries and risk factor study released by The Lancet Neurology in 2021 stated that 3.4 billion individuals (43%) across the globe lost years of healthy life due to neurological conditions. The central and western sub-Saharan African regions are suffering from highest burden of neurological disorders [3]. Earlier mortality statistics were the primary criteria undertaken for assessing the severity of a disease by the researchers and policy makers. However, focusing solely on mortality stats underrates the estimation of substantial disability experienced in individuals due to non-fatal diseases or disorders. Substantial disability leads to the reduced quality of life because the individual has to live with the condition that influences their day to day activities[4]. Neurological or psychiatric disorders are the leading cause of disability suffering globally according to the statistics of time lived with disability instead of mortality rate[5]. According to the report of World Federation of Neurology 2023 the neurological disorders are the second highest cause of death and the figure of persons living with brain diseases or disorders is expected to double by 2050 [6]. The statistical data of National Alliance on Mental Illness (NAMI) California states that in United States suicide due to mental health conditions is the second leading cause of death among the individuals of 10 to 34 years' age group [7]. Although number of multi-targeted allopathic and alternative treatment methods proposed and utilized for the cure of neurological diseases or disorders yet completely effective treatments are not available. This urges for the urgent need to develop the efficient targeted treatment that can work with the deep understanding of health challenges [8]. Keeping in mind about dealing with health challenges and imperative need of developing the efficient treatment nowadays the significance of adopting the targeting based novel technologies such as nanotechnology, proteomics and genomics are highly recommended [9]. Proteomics and genomics include the identification, quantification and characterization of complete set of protein or gene present within the biological sample such as cell, tissue or organ at a given time. These approaches plays important role in understanding the biological processes (cell signaling, metabolism & disease mechanism) at molecular level and therefore identifying potential targets for drug discovery[10]. Klotho protein in brain which is predominantly found in choroid plexus, purkinje EC cells and hippocampus neurons act as cognition enhancer and thus plays significant role in certain neuronal disorders[11]. The aim of this review is to explore the molecular mechanism of Klotho protein and the potential therapeutic interventions targeting the expression of klotho in neuronal disorders.

STRUCTURAL INSIGHT OF KLOTHO PROTEIN

The Klothogene was identified in 1997 and the name was adopted based on the ancient mythological Greek goddess "*Clotho*" that means "who weaves the thread of life". Initially the gene was discovered in transgenic mice during experiments wheregenetic mutations in Klotho protein indicated significant effects on aging [12]. This anti-aging protein plays protective role in the neurons, kidney and other organs of biological system and exhibits their action by altering various molecular and cellular pathways associated with diseases [13]. Individuals lacking the klotho gene may experience age related abnormalities such as gait issues, cognition impairment, reduced myelination in nerves, neurodegeneration in hippocampal region of brain, osteoporosis etc. [14] The Klotho protein is primarily found in the tissues that regulate calcium level, distal convoluted tubules of kidney, parathyroid hormone producing cells and choroid plexus region of brain [15]. The klotho gene is located on the chromosome 13 in humans, chromosome 12 in rats and chromosome 5 in mice. The klotho gene consists of 5 exons which encodes type I single pass trans-membrane glycoprotein. In humans, the klotho gene is translated into three protein forms i.e. α-klotho, β-klotho and ϒ-klotho. Although the structure of human and animal klotho protein is similar but in case of animals such as mice the trans-membrane form is more predominant, whereas, secreted form is more abundantly found in humans [16]. The structure of trans-membrane klotho protein encodes a 1014 amino acid protein in animals and 1012 amino acid protein in humans along with functional domains (i) N-terminal signal sequence: This allows the secretion of protein from the cells. (ii) Internal repeats (KL1 and KL2): These are the regions within the protein that resembles glycosidases and are essential for the enzymatic action of klotho protein. The repeat contains a proteolytic cleavage site that is involved in interaction with other proteins. (iii) Trans-membrane domain (iv) Short intracellular domain: involved majorly in intracellular signaling processes [17]. The structure of secreted klotho protein encodes a truncated 550 amino acid proteins and consists of N-terminal signal sequence and KL1 repeat unit only. TheKL2 repeat unit, trans-membrane and intracellular domain is absent in the secreted form of klotho protein. Apart from this the secreted form is involved in several physiological processes such as age related disorders, oxidative stress response, vitamin D metabolism and insulin sensitivity etc. [18, 19]

ROLE OF KLOTHO PROTEIN IN CNS

Several *in vivo* experiments show thatα-klotho protein is essential for maintaining the normal functioning of the brain. In animals with reduced level of α-klotho protein, different brain regions, majorly hippocampus show signs of degeneration leading to cognitive impairment, motor disorders, cholinergic dysfunction and neurodegenerative diseases such as Alzheimer's disease or Parkinsonism etc. On contrary, the higher concentration of α-klotho protein suggests neuro-protective effect that is demonstrated by improved cognition and regeneration of neurons in experimental animals [20].

EXPRESSION OF KLOTHO PROTEIN IN CHOROID PLEXUS

The choroid plexus is a specialized tissue of brain which protects the brain and spinal cord, acting as a barrier between the cerebrospinal fluid and bloodstream. The choroid plexus is the major site of cerebrospinal fluid production and responsible for the continuous regeneration and circulation of this fluid. It also serves as a source of secreted soluble factors for cerebrospinal fluid [21]. α-klotho protein is present in highest concentration in the epithelial cells of choroid plexus and can be confirmed by its detection in CSF. The declined level of klotho protein in cerebrospinal fluid has been observed in aged patients suffering from neurodegenerative diseases and multiple sclerosis [22].

EXPRESSION OF KLOTHO PROTEIN IN OTHER BRAIN REGIONS

The brain regions such as cortex, hippocampus and others, contain α-klotho mRNA that indicates the presence of this protein in neurons as well as in oligodendrocytes. These brain regions produce its own klotho protein separately and is independent on the protein produced in the blood stream or in choroid plexus. The concentration of soluble α-klotho protein is relatively higher in brain as compared to the membrane bound α-klotho protein that indicates the soluble form plays crucial role in the brain. Although the significance of membrane bound α-klotho protein in brain is not clear but it is majorly associated with the protective role in kidneys [23, 24]. The significance of klotho gene in cognition enhancement is supported by a study in humans where KL-VS variant of the klotho gene was tested and the results indicated memory boosting and increased levels in brain of heterozygotes whereas opposite results were obtained in homozygotes [25].

IMPACT OF KLOTHO PROTEIN ON NEURODEVELOPMENTAL PROCESSES

In the pathophysiology of neurological conditions, mitochondrial dysfunction is a critical factor as it is required to regulate the neuronal branching, impulse generation and maintaining the strength and stability of synapsis in the CNS [26]. During the aging process, myelin covering undergoes transformation, that ultimately leads to impairment of neuronal functioning and the klotho protein plays aprotective role in this process. The level of klotho protein declines with age and causes cognition related issues. Several animal studies indicated that the klotho protein promotes the cell maturation in myelin production called as oligodendrocytes by influencing certain signaling pathways. It has also been noticed that impairment in myelin is caused due to the decline in the level of myelin related proteins and genes, immature oligodendrocytes and abnormalities in nerve cell connections [12]. An *in vitro* study on human oligodendrocytes hybrid cell lines MO3.13 stated that klothodecreases cell growth and increases cell

differentiation via affecting ERK and Akt signaling pathways. The significant observations marked by the changes in the gene activity patterns similar to those seen in conditions such as heart diseases, cancer, stress and aging. The lower level of klotho protein in brain tumors indicates that boosting up its level can be a potential treatment option for brain and other type of cancers [27]. A neuroprotective cold shock protein RBM3 (RNA binding motif 3) improves neurological function by protecting the brain cells from ischemic brain damage. The action of RBM3 increased by an endocrine hormone fibroblast growth factor 21 (FGF21) that can easily cross the blood-brain-barrier (BBB) and stimulates the action of transmembraneβ-klotho protein (mostly absent in adult's brain) expressing neurons in the hypothalamus. RBM3 works in combination with β-klothoprotein that facilitates the FGF21 to perform its action properly. The concentration of RBM3 and β-klotho protein is higher in prefrontal cortex of young brain (such as infants). The β-klotho protein allows the binding of FGF21 to its receptor site named as FGFR1 present on the cell surface which further leads to the activation of PI3K/Akt signaling pathway that promotes cell survival and decline the cell apoptosis rate [28]. *In vivo* studies indicated that treatment with recombinant human (rh) FGF21 against ischemic brain injury showed positive outcomes such as decreased brain damage and improved memory and motor activities. The effect of rhFGF21 partially reversed when the function of β-klotho protein inhibited, suggesting that β-klotho protein is a critical component for FGF21 to exert its neuroprotective action [29]. The influence of klotho on adult hippocampal neurogenesis and cognition was examined in hippocampus of klotho knock-out mice, klotho knock-out/Vitamin D receptor mutant mice, and model of local klotho hippocampal knockdown using techniques like PCR and immunofluorescence etc. The study showed that klotho is expressed in the granular cell layer of dentate gyrus in the adult hippocampus along with increased neurogenesis in klotho knock-out mice only. It indicates that downregulation in the level of klotho will also cause downfall in the hippocampal neurogenesis and cognitive performance. Further experiments with recombinant Klotho leads to increase in the proliferation of neural progenitor cells in the hippocampus [30]. The impact of an anesthetic named as sevoflurane, majorly used in pediatric surgeries on the developing brain was studied in newborn rats. The study stated that the exposure to 3% sevoflurane boost up the mRNA of klotho protein in hippocampus of neonatal rats. It was concluded from the study that klotho protein protects the brain cells from the harmful effects of sevoflurane by reducing the neuronal stress and chances of mitochondrial injury [31]. Klotho protein enhances cognition and neural elasticity by modulating the signaling pathway of age related brain pathology in aging mouse models of neurodegenerative disorders [32].

ROLE OF KLOTHO PROTEIN IN NEUROPSYCHIATRIC DISORDERS

Schizophrenia is a chronic progressive neuropsychiatric disorder characterized by persistent delusions, hallucinations, disorganized thinking, behavior, limited speech, restricted expression of emotions, memory dysfunction and social withdrawal etc. [33]. Cognitive impairment is the main feature in schizophrenia and the effect of klotho protein on cognition in schizophrenic patients was studied. The study indicated the high levels of klotho protein was present in patients suffering from schizophrenia as compared to the healthy volunteers and the elevation was associated with better cognitive performance. The exact mechanism behind the increased level of klothois unclear but can be correlated with antioxidant mechanism of klotho produced in response to the stress condition or due to the malfunctioning of certain receptors in the brain which trigger the production of klotho [34]. In another study, the serum levels of klotho and FGF23 were estimated in schizophrenic patients and healthy volunteers. Higher levels of both klotho and FGF23 were found in schizophrenic patients as compared to the healthy group that suggests that klotho or FGF23 serve as crucial targets for the management of schizophrenia [35]. A study on the impact of klotho protein and other neurotrophic factors such as BDNF, GDNF and NGF on cognition in schizophrenic patient and healthy individuals showed that lower levels of klotho protein and neurotropic factors contributed to the cognitive decline in schizophrenic patients [36]. The effect of miRNA-339-5p which regulates klotho gene expression was studied in schizophrenic patients and healthy individuals. The results of study showed the klotho gene was more active whereas miRNA-339-5p was less active in schizophrenic patients than control group indicating inverse correlation between the two factors [37]. Depression is another neuropsychiatric disorder which is also associated with cognitive impairment. Klotho protein known for its anti-aging properties work to maintain cognition in depressive patients by two main mechanism i.e. oxidative stress and glutamate transmission related. The klotho protein regulates the oxidative stress and inflammation by antioxidant response by activation of Nrf2 and NF-κβ as well as inhibits the production of pro-inflammatory cytokines [38]. Glutamate is an excitatory neurotransmitter and its decreased level leads to mood alterations in depressed patients. Klotho modulate glutamate neurotransmission by increasing the expression and activity of excitatory amino acid transporters (EAATs) which transports glutamate and further regulates neuronal excitation and synaptic transmission. Klotho protein also influences the NMDA receptors and therefore boost up memory and improves synaptic transmission [39]. The post-mortem brain samples of an epileptic individual indicated the decreased level of klotho protein in hippocampus region [40].

NEUROTRAUMATIC DISORDERS

Cerebral infarction or stroke generally arises due to ischemic injury to the brain tissues that impair brain functioning. The protective role of klotho protein against impaired neurological functions in rats suffering from cerebral ischemia was reported in a study. The alleviated neurological function mediated through the overexpression of klotho that lowers the expression of AQP4 and inhibits the activation of P38-MAPK [41].

NEURODEGENERATIVE DISEASES

Klotho protein can protect the neurons against age related neurodegenerative diseases. The level of secreted klotho (s-KL) protein was investigated in the triple transgenic Alzheimer's disease mice model. The researchers concluded that with aging the level of secreted klotho protein was decreased in prefrontal cortex, cerebral cortex and hippocampus region of mice brain. Klotho protein act by antioxidant mechanism where it protects the neurons of hippocampus region from amyloid formation and glutamate toxicity. Klothoprotein protects the CNS from amyloid toxicity by interacting with soluble amyloid precursor protein (APPsβ). The presence of klotho protein is also necessary for the maturation of oligodendrocytes as well as to maintain the myelin integrity [42]. Multiple sclerosis is characterized by demyelinated lesions throughout the brain, spinal cord, optic nerves. The lesions result from the immune mediated attacks against protective covering of nerve fibers i.e. myelin sheath causing disruptions in nerve signaling [14]. In eye klotho protein is present throughout the retina with high concentration in retinal ganglion cells. With aging decline in klotho protein cause degeneration of retinal pigment epithelium and the increased expression of klotho protein act as protective against diabetic retinopathy [43]. Another condition optic neuritis which is an expression of multiple sclerosis affects the myelinated part of retinal ganglion cell axons particularly in pediatric population. *In vivo* studies indicated klotho protein promotes re-myelination, repairs myelin and easily crosses the blood brain barrier (BBB) and thus produces its action [44].

INTRACELLULAR SIGNALING NETWORKS

The klotho protein has gained significant attention in the area of neurological disorders due to its emerging role in regulating various signaling pathways implicated in neuroprotection, synaptic plasticity, and cognitive function [45, 46]. Several intracellular signaling pathways have been linked to the neuroprotective effects of klotho in neurological disorders that are discussed and depicted in Figure 1.

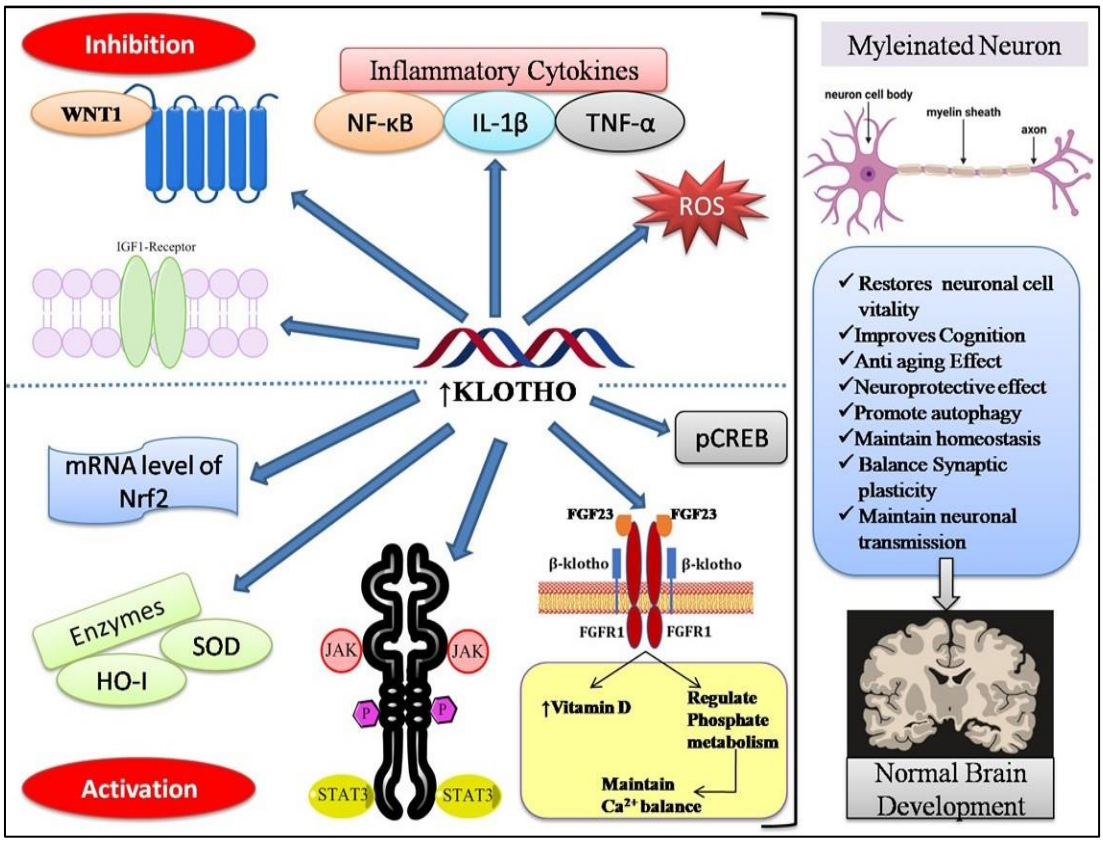

Figure 1: The diagram illustrates the multifaceted role of Klotho in regulating neuronal health and brain development through various molecular pathways. Klotho inhibits inflammatory cytokines, including NF-κB, IL-1β, and TNF-α, thereby reducing oxidative stress (ROS). It also suppresses WNT1 signaling and enhances the expression of neuroprotective factors such as pCREB. Activation of pathways like JAK/STAT3 promotes the expression of antioxidant enzymes (SOD, HO-1) and upregulates Nrf2 mRNA levels, contributing to cellular protection. Klotho enhances the regulation of phosphate metabolism through FGF23 and Vitamin D, essential for maintaining calcium balance and supporting neuronal transmission. These combined effects contribute to neuronal vitality, cognitive function, synaptic plasticity, and the overall development of a healthy brain.

Wnt Signaling Pathway

Wnt1 is a proto oncogene that acts as a crucial factor in the process of cell proliferation and maintenance as its continuous exposure boost up the aging process in cells. Klotho protein inhibits the Wnt signaling pathway and thus plays significant role in neuronal development and synaptic plasticity. The effect can be demonstrated by a study where the effect of klotho pre-treatment in different concentrations on human SH-SY5Yneuroblastoma cells affected with amyloid β toxicity was studied. The findings of the study showed klotho treatment restores neuronal cell viability by decreasing reactive oxygen species (ROS), inflammatory biomarkers i.e. NF-κβ, IL-1β, TNF-α, and inhibition of Wnt1 level in amyloid beta exposed neuronal cells. In contrast to this there was upregulation of certain enzymes such as superoxide dismutase, hemeoxygenase 1 (HO-1), phosphorylated cyclic AMP element binding (pCREB), and mRNA level of Nrf2. Klotho protein increases neuroblastoma cell viability through modulation in Wnt1/NRF2/HO-1/pCREB signaling pathways [47]. Another study showed that klotho act as tumor suppressing agent by the inhibition of Wnt1, whereas, upregulation of insulin/IGF1 signaling pathway and protein p53/21 [48]. In patients suffering from chronic kidney disease cerebral klothoproduction declines which activates the Wnt signaling affecting Wnt/β-catenin activity and leads to cognitive dysfunction [49].

FGF23 Signaling pathway

The role of FGF23 is not extensively studied in neurological disorders but it plays a significant role in the amelioration of cognition. FGF23 influences cognition through several interconnected mechanisms such as alterations in metabolism of vitamin D and calcium homeostasis [50]. Klotho protein expressed in brain also regulates vitamin D. In brain Vitamin D regulates the expression of genes involved in neuroprotection so the down regulation of FgF23 affects the vitamin D level and ultimately results in cognitive decline [51]. A research study on mice lacking FGF23 and Klotho genes reported the relationship between Vitamin D levels and cognition which suggests that it is essential to maintain the optimum level of precursor form of Vitamin D i.e. calcidiol than the active form of vitamin D i.e. calcitriol. Ensuring the adequate level of calcidiol is important for promoting delay of cognitive decline [52, 53]. Calcium is essential for neuronal transmission and synaptic plasticity in the brain and thus manages brain related diseases or disorders such as Alzheimer's disease, Parkinson's, multiple sclerosis etc. [54]. FGF23 also regulates the phosphate metabolism and the dysfunctioning of FGF23 signaling pathway and can result in hypophosphatemia which causes imbalance of calcium and ultimately leads to neurodegeneration and cognitive impairment [55].

Insulin/Insulin like growth factor 1(IGF-1) signaling pathway

Klotho protein competes for insulin/IGF1 receptor present on cell membranes and blocks the IGF-1 signaling pathway. This prevents the downstream effects that promotes aging. Apart from this klotho activates certain factors in brain that protect neuronal cell damage from oxidative stress [56]. Research suggests that promoting autophagy (process of clearing out the abnormal protein like amyloid beta from neuronal cells) can increase the neuronal cell viability and helps to fight against neurodegenerative diseases or disorders. The cell growth and the process of autophagy are controlled by PI3K/Akt/mTOR signaling pathway. This pathway is activated by IGF-1 and the over activity of this pathway hamper the process of autophagy and causes protein accumulation in Alzheimer's. The highest concentration of anti-aging klotho protein regulates autophagy by blocking IGF1 signaling pathway and reducing oxidative stress in the brain [57]. The longevity pathways such as mTOR, SIRT, IIS and IGF1 signaling pathways regulate cellular processes (such as protein synthesis, mitochondrial function, autophagy etc.) and maintain homeostasis. Several environmental and genetic factors or aging disturb these processes and possibly leads to the progression of neurodegenerative associated diseases [58]. Another study reported on *Yisuimoxibustion*, which slows down the aging by enhancing the level of klotho protein in the brain of mice and the effect is predicted to occur through the modulation of IGF1 signaling pathway [59].

PKA/CaMKII/CREB signaling Pathway

Klotho protein is known to protect neurons from oxidative damage because of its anti-aging properties. The effect of exogenously administered klotho against nigrostriatal dopaminergic pathway injury was studied in 6-hydroxydopamine rat model of Parkinson's disease. The klotho treatment showed improved recovery against the injury and the effect was mediated by the inhibition of protein kinase A, H-89 and Ca^{2+}/calmodulin dependent protein kinase II (CamKII) KN-62. Besides modulating the above mentioned signaling pathways it exerts its action by lowering the levels of malondialdehyde, reactive oxygen species, phospho-cAMP response, protein element binding (pCREB) and DNA fragmentation etc. [60]

JAK2/STAT3 signaling pathway

A study reported on the involvement of JAK2/STAT3 pathway and klotho protein in producing the neurotoxic effect through cadmium exposure experimental rat model. Cadmium exposure to rats causes cognitive impairment and the effect produced is mediated to occur through the decrease in the phosphorylation of JAK2/STAT3 in the cells and klotho protein expression [61].

NF-κB-TNF-α signaling

In vivo study reported that animals suffering from cognitive issues showed higher neuronal inflammation particularly in frontal cortex region, increased level of inflammatory markers such as NF-Kb and TNF-α in cerebrospinal fluid and lowered level of klotho protein [62]. In another study the role of major inflammatory factors i.e. tumor necrosis factor, nuclear factor kappa B and klotho protein were studied in individuals with temporal lobe epilepsy related to

hippocampal sclerosis. It has been noticed that the expression of TNF-α and NF-κB1 was elevated and the expression of klotho protein was declined causing inflammation in the hippocapal region of brain [40]. A study conducted to investigate the effect of klotho and lingaliptin treatment on immune cells called as peripheral blood mononuclear cells in Alzheimer's patients. The inflammatory markers (IL-1β, IL-6, TNF-α etc.), signaling proteins (PKC PKCε, pCREB, and Wnt1) and microRNAs (miR-29a & miR-195) were measured in these patients after treatment. The results of the study indicated that the treatments reduced the levels of inflammatory biomarkers along with downregulation of Wnt1 and upregulation of pCREB and microRNAs [63]. A study examined the impact of klotho protein levels in ischemic brain injury. Increased klotho improved the functioning of brain in ischemic stroke like conditions by influencing the anti-inflammatory signaling cascade [64].

EPIGENETIC MECHANISM OF KLOTHO PROTEIN IN NEUROLOGICAL DISORDERS

Several medications and natural compounds exhibit their neuro-protective action through epigenetic mechanism especially by influencing the mitochondrial function, preventing demyelination of neurons and neuro-inflammation [65]. The level of anti-aging Klotho protein is lower in women than men. Literature also indicated that the cases of neurodegenerative diseases like AD and PD are more likely to be found in women as compared to men and the major contributing factors includes certain biological such as changes at hormonal level and other social factors [14]. Klotho regulates calcium and phosphorous homeostasis and influences the level of vitamin D through its action on FGF receptors. The deficiency of vitamin D is associated epigenetically with down regulation of klotho, affecting the overall homeostasis which leads to the progression of certain neurological diseases or disorders [66]. In case of neuro-inflammation like in multiple sclerosis there is elevation in the level of tumour growth factor β causing hypermethylation of klotho gene promoter region through the activation of enzyme DNA methyltransferase. This epigenetic alteration leads to the inhibition of gene transcription step further decline in klotho protein level and therefore contributes to cognitive decline [67]. A study conducted on military veterans to find out the relationship between the klotho gene polymorphisms as well as DNA methylation in klotho gene and post-traumatic stress disorders. The results showed that klotho gene is linked to changes in DNA methylation at particular site and the severity of PTSD can affect the gene's impact on the level of methylation. The higher methylation level is associated with the lower inflammation in the body and the interaction between the gene and PTSD affects inflammation level through the alterations in methylation[68].

THERAPEUTIC INTERVENTIONS TARGETING KLOTHO

Klotho Supplementation

Klotho is an aging suppressor protein whose expression declines throughout the aging process as well as in stress like conditions. The exogenous administration of klotho can improve the learning or memory retaining performance in individuals by modulating several intracellular signaling pathways [69]. The beneficial effects of klotho supplementation can be demonstrated by various preclinical and clinical studies which are as follows.

Antioxidant Effect

The exogenous supplementation of klotho protein enhances antioxidant defenses in astrocytes and ubiquitin proteasome activity in neurons. This activity is demonstrated through an *in vitro* experimentation where the neuronal and astrocytic cell cultures were exposed to klotho protein and the results showed increased antioxidant activity in the neuronal cells by decreasing the phosphorylation of insulin/insulin like growth factor signaling pathway [70].

Nootropic Effect

The over expression of klotho protein in certain neurodegenerative and neuropsychiatric diseases is known to improve cognition. An animal study reported that klotho protein exhibits nootropic action by lowering the level of amyloid beta in the mice brain therefore beneficial for the management of neurodegenerative diseases [71]. A cross sectional study reported where the relationship between serum klotho and cognitive performance was examined among the adult population of U.S. The findings of the study suggest concentration of klotho protein relatively affects the cognition among the selected individuals and can be maintained by proper supplementation of klotho [72].

Anti-inflammatory Effect

Depression is the major risk factor for the progression of dementia and its associated neurodegenerative disorders. Research findings indicated chronic depressive states often related to dementia and generally linked through common pathways such as dysregulation of HPA axis, shrinkage of hippocampus, decline in the level of nerve growth factors, and due to oxidative stress or overexpression of certain inflammatory responses [73]. PPAR-ϒ activation reduces inflammation by inhibiting the pro inflammatory gene expressions. A study reported on the role of FGF21 in traumatic brain injury in an *in vitro* experimentation on human brain cell lines. The study demonstrated the neuroprotective role of FGF21 exerted through the activation of peroxisome proliferator activated receptor-gamma (PPAR-ϒ), increased expression of β-klotho level and maintaining integrity of blood brain barrier (BBB) [74].

PHYTOCONSTITUENTS MODULATING KLOTHO IN NEURONAL DISORDERS

Some phytoconstituents explored for their neuroprotective action by modulating the expression of klotho protein are

depicted in Figure 2. Allicin is a thioester of sulfenic acid and an active constituent of *Allium sativum*. Itis responsible for the odour of garlic and possess therapeutic properties such as nephroprotective, neuroprotective, antihyperlipidemic, anti-inflammatory, antioxidant, spasmolytic etc. In majority of neurological disorders there is neuronal inflammation, mitochondrial dysfunction or demyleination of neurons. Allicin is reported to repair mitochondrial dysfunction of neurons through inhibition of mTOR signaling pathway which in turn increases the expression of klotho protein therefore promotes neuronal longevity [75]. Boswellic acid which is a pentacyclic triterpene found in the gum resin of *Boswellia* species is a promising protective agent for the management of brain diseases. Several preclinical studies provide evidence for the beneficial effect of boswellic acid against neurodegenerative diseases and age related disorders [76]. A recent study reported on neuro protective effect of polymeric micelles of boswellic acid-selenium in traumatic brain injury indicated the beneficial results through up regulating the expression of BDNF and klotho along with inhibitory effect on excessive neuronal excitation induced by glutamate as well as dysregulation in the expression of miR-155 and miR-146a signaling pathways [77].

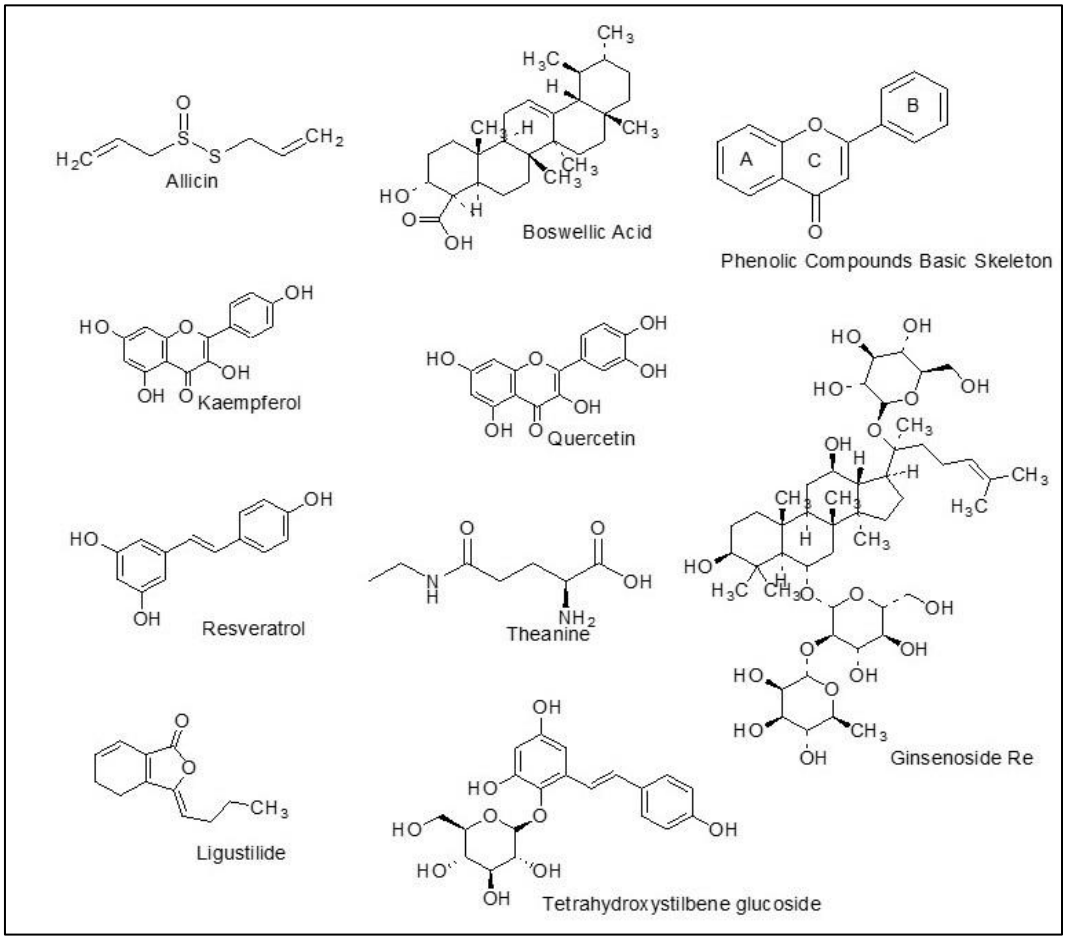

Figure 2: The chemical structures of various phytoconstituents with neuroprotective effects, known to modulate Klotho expression and signaling pathways, are depicted. These phytoconstituents are known to exert neuroprotective actions by enhancing antioxidant defenses, reducing inflammation, regulating calcium homeostasis, and promoting neuronal health, thereby contributing to the overall protection and maintenance of brain function.

Cynaras colymus commonly known as artichoke is traditionally known for its beneficial effects against certain health conditions because of the richness of several bioactive phytoconstituents majorly phenolic compounds. The methanol extract of this plant was examined for neuroprotective effect against diethylnitrosamine induced toxicity in experimental animals. The extract was found to increase the expression of klotho protein and peroxisome proliferator activated receptor gamma (PPARγ) through antioxidant and antiapoptotic mechanism thus producing neuroprotective action [78]. Ginsenoside Re (GRe) is a protopanaxatriol type saponin obtained from the plant *Panax ginseng* that is reported to promote longevity. A study reported neuro protective effects of ginsenoside Re in klotho deficient experimental animals. The findings of study suggest that GR reduced aging through antioxidant mechanism by activating nuclear factor erythroid 2-related factor 2 signaling pathway which significantly elevated the action of glutathione peroxidase [79]**.** Kaempferol is a flavonoid abundantly found in vegetables and fruits. The presence of four hydroxyl groups in the structure of kaempferol is responsible for its antioxidant potential. The neuro protective and memory boosting action of this polyphenolic phytoconstituent was evaluated in streptozotocin induced neurodegeneration model of Alzheimer's disease. Kaempferol increased cognition and

improved synaptic plasticity by increasing the expression of antiaging klotho protein [80]. Ligustilide is a natural phthalide found in highest concentration in *Angelica sinensis* and *Ligusticum chuanxiong.* This phytoconstituent is lipophilic in nature therefore can easily penetrate into the blood brain barrier (BBB) and produce the targeted effect. In preclinical studies ligustilide showed neuroprotective effects against ischemic brain injury, memory impairment, neuro-inflammation etc. In another study its anti-amnesic studies reported against double transgenic mouse model of Alzheimer's disease and the significant beneficial results reported with increase in the expression of amyloid precursor protein and klotho protein along with the inactivation of IGF-1/Akt/mTOR signaling pathway [81]. Quercetin is an antioxidant flavonol that is chiefly found in fruit berries and green leafy vegetables. This phytoconstituent is known to exhibit number of biological activities such as anti-inflammatory, anticancer, mitochondrial biogenesis, free radical scavenging etc. An *in vivo* study reported on the effect of treatment with quercetin and vitamin D on neurological functions in animal model of chronic kidney disease. The biochemical tests indicated the increased expression of brain derived neurotrophic factor (BDNF) and klotho protein as well as decline in certain parameters such as oxidative stress, fibrosis and apoptosis that leads to the neuro and nephro protective action [82]. Resveratrol belongs to stilbene class and is found abundantly in berries (*Vaccinium spp.)*, grapes (*Vitis vinifera)* and red wine. The constituent has significant antioxidant property and has anti-aging potential. In an animal model of aging, treatment with resveratrol increased neuronal cell vitality rates by increasing the activity of superoxide dismutase (SOD) and catalase enzyme and led to up regulation in the expression of klotho protein [83]. Selaginellins are group of pigments having diverse polyphenolic structural skeletons and was first reported in *Selaginella sinensis.* In animal experiment, the neuroprotective action of selaginellin was investigated in L-glutamate induced rat PC12 cell death and the findings of the study indicated the beneficial action is driven through antioxidant and anti-apoptotic mechanism by the up regulation in the expression of klotho gene [84]. Tetrahydroxystilbene glucoside (TSG) a constituent from roots of a Chinese herb *Polygonatum multiflorum*is reported to possess neuroprotective action in age related disorders such as dementia by ameliorating learning and memory abilities. This phytoconstituent act as free radical scavenger and reported to exhibit its neuroprotective action by activating phosphorylation of PI3K/Akt signaling pathway. The influence of tetrahydrostilbene glucoside on the level of klotho and insulin like growth factor 1 (IGF) and its subsequent effect on cognitive functions of brain was investigated in the study. The study suggests TSG up-regulated the level of klotho and inhibited the IGF-1, thus, producing anti-aging action [85]. Theanine an amino acid most abundantly found in tea (*Camellia sinensis)* is a rich source of antioxidants that improves cognition and promotes longevity. Theanine tested for its cognitive boosting activity in animal model that lack klotho gene. The study showed that theanine significantly improves memory function in experimental animals by activating the phosphorylation of JAk2/STAT3 which in turn leads to increase in the expression of klotho[86].

EFFECT OF VITAMIN D SUPPLEMENTATION ON LEVELS OF KLOTHO PROTEIN

A double blinded randomized placebo controlled clinical trial was conducted to investigate the effect of vitamin D supplementation on klotho protein. Total 90 participants of age group over 60 years with vitamin D deficiency were administered with vitamin D 50000 IU/week, provided as cholecalciferol, for 12 consecutive weeks. The results of trial studies indicated the beneficial role of vitamin D supplementation as it prevented the reduction in concentration of klotho protein in plasma [87].

KLOTHO GENE THERAPY

An interventional non randomized study was conducted to evaluate the safety of novel proprietary CNS gene transfer method to deliver AAVhTert and klotho genes to five patients suffering from mild to moderate dementia. The therapy proved to be safe as the data of clinical responses didn't indicate any serious side effects. One year follow up reports of patients showed significant improvement in the symptoms of dementia suggesting the gene therapy as promising approach for the management of dementia [88]. The impact of intra cerebroventricular injection oflentiviral vector which encodes the transmembrane form of mice Klotho cDNA to APP/PS1 transgenic mice of Alzheimer's disease was studied. The findings of this study suggested that the treatment enhance the level of klotho protein in brain and promotes the clearance of amyloid beta through the process of autophagy and thus exhibits improved cognitive activity in AD mice model [89].

CHALLENGES AND FUTURE PROSPECTS

The antiaging Klotho protein holds tremendous potential as a safer alternative over conventional treatment strategies for neuronal disorders. The existing research studies show the neuroprotective effect of klotho protein through supplementation of certain plant based constituent or gene therapy approach that restores the normal levels of klotho protein. The investigation of comprehensive safety profile associated with klotho supplementation is not yet reported clearly thus needed to explore in order to validate the safety profile [16]. Most of the studies reported are preclinical in nature and only a few clinical trial studies have been conducted and reported, therefore, can't provide any potential toxicity related information [90]. A number of plants and their constituents are reported for the management of neuronal disorders but in most of the cases they often failed to reveal the exact mechanism of action at cellular or molecular level. Therefore, it is also important to significantly elucidate the specific molecular mechanism through which klotho supplements exerts the neuro-protective action. The scientists can predict the possible role of cellular signaling pathways by adopting *in silico* approach and later on confirmation of computational

results through *in vitro* or *in vivo* experimentation [91]. All the above discussed challenges highlight the need of exploring the klotho based novel treatment strategies for future researchers to address the existing research gaps with improved outcomes.

CONCLUSION

Neurological disorders have high prevalence rate affecting the quality of life of a person. Conventional existing treatment has certain limitations such as side effects, withdrawal symptoms, dependence, suicidal thoughts, reduced efficacy, delayed onset of action, partial response etc. The challenges associated with current treatment urges the need of adopting the innovative, effective and molecular targeted based treatment. Molecular targeting based treatment focus to address the biological understanding of neuronal disorders at molecular level including modulating the specific receptor, transporter system or gene expression which further activate or inhibit certain signaling pathways involved in the normal functioning of the brain. Klotho is an antiaging protein that is normally found in certain areas of brain such as choroid plexus, purkinje cells of cerebellum, cortex, hippocampus, substantia nigra, medulla oblongata etc. The deficiency of klotho protein leads to cognitive decline, increased oxidative stress, apoptosis, demyelination of neurons, and mitochondrial dysfunction of neurons etc. that plays important role in the progression of majority of brain diseases and disorders. Expression of klotho protein impacts the overall functioning of brain through activation and inhibition of certain neuronal signaling pathways. Increased expression of klotho protein inhibits the activation of WNT1, IGF-1, reactive oxygen species and pro-inflammatory biomarkers (NFκB, TNF-α, IL-1). Increased expression of klotho protein also leads to the activation of anti-oxidant enzymes, JAK2/STAT3 phosphorylation, FGF23 activation, Nrf2 antioxidant pathway. Several therapeutic interventions such as supplementation of phytoconstituents are reported in literature that upregulate the expression of klotho protein and alleviate neuronal conditions. Although some studies report the beneficial effects of increased klotho in neuronal disorders, the study in this area is relatively less explored. There is a future need to explore this field in order to provide valuable insight for effective treatment for a broad spectrum of neuronal disorders.

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AUTHOR CONTRIBUTIONS

Gulsheen Panesar: conceptualized the idea and edited the work

Shivani and Nidhi Sharma: prepared the first draft;

Sharad Sardana: edited the work

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