

# ROLE OF CHRYSIN IN NEUROLOGICAL DISORDERS

## INTRODUCTION

Neurological disorders is a common problem in neurology practice. Over the past decades, interest in this group of diseases has increased in research studies. These diseases are a huge health burden around the world. It affected 1.5 billion people worldwide. Some of the neurological disorders are: epilepsy, Alzheimer disease, Parkinson disease, multiple sclerosis, gliomas, traumatic brain injury, ischemic brain injury are included in global burden of disease (GBD) study and rank in top 50 causes of disability adjusted life years (DALYs) [1].

We should focus on neuroprotective agents because drugs are not much beneficial due to its large side effect. As there is growing evidence that flavonoids have beneficial effects on the mind, polyphenols may be seen as attractive targets for drug design and discovery.

Flavonoids are a class of secondary metabolites that contains large groups of low molecular weight polyphenolic compounds, widely found in fruits, Vegetables, beverages. Flavonoids are associated with broad spectrum of Health promising and found in various nutraceutical, pharmaceutical, medicinal applications. They are found in abundance over 8000 flavonoids has known [2]. According to nutritionists, average value of flavonoid to be taken by human is between 1-2g / day. They display large range of structures and ecological significance and have major biological activities. One of the most important flavonoid to study is chrysin (5,7 dihydroxy flavone).

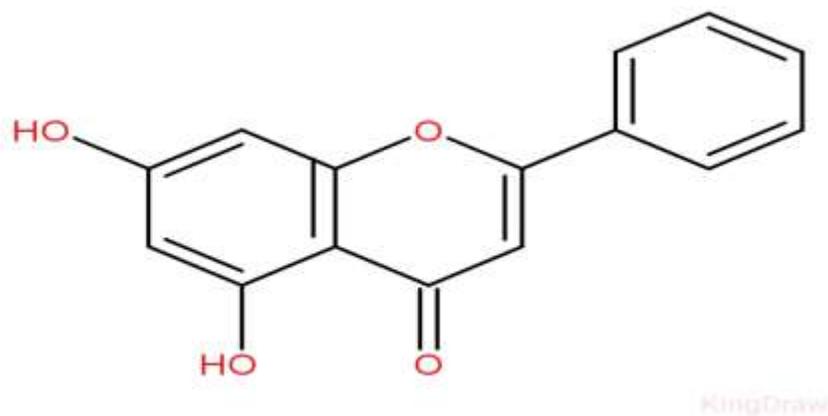
## CHRYSIN

Chrysin, also known as chrysinic acid belongs to the class organic compounds known as flavones. It is mainly found in honey, propolis and many plant species such as: *pelargonium crispum*, *Passiflora incarnata*, *oroxylen indicum*.

It possesses various pharmacological properties such as – anti-inflammatory, pro-inflammatory, anti-asthmatic activity, anticancer, anti- hypercholesterolemia activity, cardio protective activity, Reno protective activity [3] and has been used as traditional medicine from ancient times. Chrysin is naturally occurring in several plants, fruits and vegetables. Here are some of the foods: Passion flower, Yerba Santa, Baikal scapula, Common skullcap, Honey and bee products.

## STRUCTURE

Chrysin consists of 2 fused rings, A and C and a phenyl ring B, attached to second position of C ring. It has additional hydroxyl group attached at 5 and 7 position of A ring.



## PHARMACOKINETICS

Polyphenols are not absorbed easily especially present in the form of esters, glycosides and polymers. Due to poor absorption and higher rate of metabolism and elimination takes place so they possess poor intrinsic activity. Polyphenol degrades aglycones and various aromatic acids after get hydrolyzed by intestinal enzymes. Aglycones are the cardiac glycosides which are most potent than other glycosides [4]. Naturally occurring flavonoids get conjugated (methylation, sulfation and glucuronidation) during passage across the small intestine in the time of absorption and then phase – 2 conjugation occurs which gives rise to glucuronides, sulfates, methyl conjugates and small quantities of free aglycones in liver.

To demonstrate pharmacological benefits and bioavailability of chrysin, it is necessary to understand role of efflux transporter and the fate of its metabolite. There are mainly 3 transporters involved in the transport of chrysin conjugates: a) MRP2 b) BCRP c) ABC

a) MRP2 (multidrug resistance associated protein 2) also known as ABCC2, is a withdrawal efflux transporter that transports anions, including drug conjugates and conjugated bilirubin. It is mainly present in tissues of liver, kidney and placenta. Studies suggests that transport of chrysin metabolite in caco-2 cells was done by MRP2 [5]. These conjugates may be hydrolyzed by sulfatases and glucuronides to chrysin after get efflux into small intestine. Caco-2 cell line reported favorable membrane Transport properties was possessed by chrysin. However large amount of unchanged chrysin in stool samples shows chrysin has poor intestinal absorption.

b) BCRP (breast cancer resistance protein) is also one of the important efflux transporter of ATP binding cassette (ABC) family of protein for phase 2 metabolites located at apical membrane of enterocytes and hepatocytes. It is also known as ABCG2.

c) ABC transporter also involved in transport of chrysin conjugates.

Study claimed that excretion of chrysin glucuronide and sulphate could be reduced by treatment of anion inhibitor MK-571 in Caco-2 cells. This suggests that MRP2 helps in efflux of chrysin conjugates up to 71% - 20% inhibition of efflux of chrysin glucuronide and sulphate [6]. Therapeutic potential, bioavailability of

chrysin and other Flavonoids are not effective due to poor absorption, rapid metabolism and elimination. The plasma concentration of a 400 mg oral dosage of chrysin is very low. Protein binding was found to be >99%. Chrysin ranging from 300- 625 mg reported as safe and enough efficient. Various attempts we're taken to increase bioavailability of chrysin by use of liposomal chrysin, nanoparticles of chrysin and synthetic analogues of chrysin [7]. The best flavone with high oral absorption, bioavailability and increased metabolic stability and increased intestinal transport was found to be 5,7 dimethoxy flavones.

The bioavailability of oral chrysin was found to be very low and high concentration of its metabolites in body fluids (plasma, bile) was seen. It concludes that once chrysin absorbed in intestine, it might be conjugated very rapidly with glucuronide and sulfuric acid. Due to chrysin interactions with CYP enzymes and BCRP, it was seen that chrysin was also helpful in pharmacokinetics of other compounds such as: caffeine, paracetamol.

### 3) PLAUSIBLE NEUROPROTECTIVE MECHANISM OF CHRYSIN

#### 3.1) Chrysin as antioxidant

Antioxidant are the substances which helps our cells to fight against free radicles. They are the molecules that can be oxidized and reduce key molecules. They have an efficient process for either excretion or reduction (such as glutathione). Reaction oxygen species (ROS) are free radicles which may cause damage to DNA, RNA and proteins and are byproducts of cellular oxidative metabolism and can cause cell death. Examples includes peroxides ( $H_2O_2$ ), hydroxyl (OH), superoxide ( $O_2^-$ ). It is mainly produced by mitochondria during arachidonic metabolism like  $O_2^-$  can formed by cellular respiration by lipoxygenase (LOX), Cyclooxygenase (COX).<sup>7</sup> Oxidative stress is major cause which is responsible for several diseases like cancer, diabetes, metabolic disorders, cardiovascular diseases. It is mainly imbalance between reactive oxygen species and oxidant defense mechanism of a cell or tissues.

One study shows that Chrysin due to its antioxidant properties it helps in reduction of oxidative stress which is due to increased lipid peroxidation results in functional degradation of tissues [8]. Feedback mechanisms on the antioxidant system were also studied by administration of chrysin at dose of 100mg/kg which results in decrease in formation of leucocytes in gamma – irradiated mice. It is also helpful in preventing the direct oxidative damage induced by metals by the process of chelation [9]. Chrysin also show reduction in brain derived neurotropic factor (BDNF) in mice *in vivo* and decreased oxidative stress [10].

#### 3.2) Chrysin as anti-inflammatory

An inflammatory substance has the ability to reduce pain or inflammation. A proinflammatory cytokine is a type of signaling molecule produced by activated macrophages or helper T-cells and involved in up regulation of inflammation.

They include interleukin (IL-1) (IL-2) (IL-18), tumor necrosis factor (TNF-alpha), interferon gamma (INF $\infty$ ). Researches done on flavonoid (chrysin) shows that chrysin activates mRNA expression in PPAR $\gamma$  on macrophage and could increase expression of PPAR $\gamma$  – dependent genes CD36, Arg 1 in primary macrophages. Chrysin inhibited macrophagic infiltration after intragastric administration at 25 mg / kg daily to the [C57BL/6] mice in experimental autoimmune uveitis (EAU).

Macrophages and neutrophils are the major mediators which were responsible for inflammation. Chrysin decreases the levels of mRNA in M1 marker genes such as - CCL3, IL-12 $\beta$  and increases the regulation of M2 marker genes such as Arg1, Ym1. It also suppresses m1 phenotype and helps in induction of m2 phenotype which acts as anti-inflammatory in peritoneal macrophages of obese mice and cultured macrophages *invitro* study. Therefore, Chrysin could induce overexpression M2 surface markers and down expression of M1 surface markers in MHC-2 cells.

### 3.3) Chrysin role in cell survival and apoptosis

Apoptosis is a form of regulated cell death that occurs in multicellular organisms. These changes include cell shrinkage, nuclear fragmentation, chromatin condensation. It is essential for embryogenesis as well as helps in protection. The lack of apoptosis influences tumor development and resistance to cancer treatment. Study claims that Chrysin promotes apoptosis induced by tumor necrosis factor (TNF)- induced apoptosis. Initial treatment with chrysin is effective in restoring different human-cancer cell lines from different tissues. Caspase 8, strongly activated by the combination of Chrysin and TRAIL, will induce apoptosis. Chrysin also helps in hypoxia by inhibiting STAT3 activation and by diminishing the effect of VEGF expression in hypoxia cancer cells. It suppresses hypoxia survival and metastatic growth of breast cancer cells [11]. Complexation of ruthenium-chrysin shows early apoptosis in MCF-7 cells by diminishing G0/G1 phase of cell cycle. It reduces cell proliferation and induce apoptosis and do act as chemotherapeutic agent [12].

STAT-1 and p21 are essential proteins that are involved in modulation and regulation of apoptotic method. Chrysin shows decrease in stages of apoptotic proteins such as Survivin after treatment with A375 cells and extend in the degree of effector caspase (caspase-3) was also observed. Thus it shows that chrysin has manageable function in causing apoptosis [13]

### 3.4) Chrysin role in Excitotoxicity

Excitotoxicity is a process in which over- activity of glutamate signaling pathway, especially N-methyl-d-aspartate glutamate (NMDA), causes abnormal levels of intracellular calcium, resulting in cell death. It has been found that GSH depletion could be reversed by initial treatment of chrysin (50 mg / kg) after lung injury. The ability of chrysin to prevent depletion of pulmonary glutathione in a model of bleomycin – induced pulmonary fibrosis is mediated, at least in part, by its antioxidant properties. Chrysin could act as ROS scavenger, accumulating reduced GSH levels in cells.

Chrysin has defensive action against doxorubicin-induced acute cardiotoxicity in rats through suppressing oxidative stress, inflammation and apoptotic tissue damage by decreasing BAX and cytochrome C expression and caspase-3 activity while growing the expression of Bcl2 levels. The stage of excitatory neurotransmitters, aspartate and glutamate, was expended through administration of chrysin in comparison to I/R group. Considerable proof shows flavonoids have specific effects on glutamate system and this contribute to their contributes to their capacity to minimize excitotoxicity [14].

### **3.5) Chrysin role in Dopamine metabolism**

Dopamine is a neurotransmitter associated with depression and has been described as important neurotransmitter that mediated movement and motivates behavior. TST is widely used to asses depressive behavior in mouse models and has been reported to act on dopamine system.

Study shows that dopamine levels in the hippocampus and prefrontal cortex are reduced in hypothyroid mice compared to controls. Chrysin is effective in normalizing dopamine levels in hippocampus [15].

### **3.6) Chrysin role in Epigenetic Modulator**

Chrysin has been shown to arrest the G1 cell cycle phase and inhibit HDAC-2 and HDAC-8. Chrysin-treated cells showed increased levels of H3acK 14, H4acK 12, H4acK16, and decreased H3 me 2Ka methylation.

It was shown that the induction of p21 by chrysin treatment was independent of p53. Chromatin remodeling of the p21WAF1 promoter induces p21 activation increases STAT-1 expression and epigenetic modulation that are responsible for cell cycle termination and cell death. Deviations in epigenetic mechanisms provide a fertile platform for tumor initiation and development. Therefore, agents that can modulate the epigenetic environment of neoplasms are useful adjuvants for anticancer therapy. Flavones appear to be anti-cancer agent because of their unique antioxidant property and ability to limit epi-target namely histone deacetylase (HDAC) [16].

Dietary flavones affect epigenetic modifications and shown anticancer properties using catalytic models DNMT and EZH2 in which plant flavones are hydrogen bonded to both ends of DNMT and EZH2 catalyst bags. Epigenetic studies show the activity of DNMT enzyme is reduced and cytokine base hypermutation in DNA is reversed [17].

**4)ROLE OF CHRYSIN IN DIFFERENT NEUROLOGICAL DISORDERS****4.1) Chrysin role in epilepsy**

Sr. No.	Dose and Route of administration	Experimental model	Animal / Cell lines	Outcome	Reference
1.	Chrysin-loaded poly (lactic-co-glycolic acid) nanoparticle (5 and 10 µg/ml)	PTZ induced kindling (35 mg/kg; i.p.)	Wistar rats	Anti-apoptotic, antioxidant	<a href="#">Zhang et. al. 2021</a>
2.	Chrysin isolated from <i>Passiflora incarnata</i> (150, 300, and 600 mg/kg; i.p.)	PTZ induced kindling	Male swiss albino mice	Anti-epileptic, Anti-depressant	<a href="#">Singh B et. al. 2012</a>
3.	Chrysin isolated from ethanolic extract of <i>pyrus pashia</i> fruit (100mg, 200mg, 400mg/kg)	MES and PTZ kindling (60mg/kg; i.p.)	Foster albino male rats	Anti-convulsant activity	<a href="#">Sharma p et. al. 2019</a>

Zhang Y, Zhao J, Afzal O, Kazmi I, Al-Abbasi FA, Altamimi ASA, Yang Z. Neuroprotective role of chrysin-loaded poly(lactic-co-glycolic acid) nanoparticle against kindling-induced epilepsy through Nrf2/ARE/HO-1 pathway. *J Biochem Mol Toxicol.* 2021 Feb;35(2):e22634. doi: 10.1002/jbt.22634. Epub 2020 Sep 29. PMID: 32991785.

Sharma P, Kumari A, Gulati A, Krishnamurthy S, Hemalatha S. Chrysin isolated from *Pyrus pashia* fruit ameliorates convulsions in experimental animals. *Nutr Neurosci*. 2019 Aug;22(8):569-577. doi: 10.1080/1028415X.2017.1418786. Epub 2017 Dec 28. PMID: 29284373.

Singh B, Singh D, Goel RK. Dual protective effect of *Passiflora incarnata* in epilepsy and associated post-ictal depression. *J Ethnopharmacol*. 2012 Jan 6;139(1):273-9. doi: 10.1016/j.jep.2011.11.011. Epub 2011 Nov 15. PMID: 22107833.

Epilepsy is neurological disorder in which electrical activity gets disturbed in nerve cells which causes seizures. The expression of Grand mal seizure induced by PTZ in mice was enhanced by the intracerebroventricular administration of chrysin. Another study shows that treatment done with extracts of (*passiflora edulis sims*) in mice shows reduced seizure severity and immobility duration compared to vehicle control in a dose and time dependent after 15 days treatment. It also helps in to maintain the levels of serotonin and noradrenaline in brain [18].

Study also suggests that chrysin helps in decreasing oxidative stress in the cortex and hippocampus and also reduces the scores of seizures in PTZ model of epilepsy. The SOD activity and GSH-PX activity is measured using MDA and the marker of membrane lipid peroxidation. It was found chrysin treatment increased the expression levels of SOD( $P<0.001$ ) and GSH-PX ( $P<0.001$ ) and decreased MDA content which was vice-versa in PTZ controlled group. Chrysin NPs (5 or 10  $\mu\text{g/ml}$ ) balances the expression of Nrf2, HO-1, and NQO-1 in both cortex and hippocampus in PTZ model of epilepsy. It is measured with the help of RT-PCR analysis. Similarly, increased levels of mRNA was shown in hippocampus by treatment of chrysin NPs (5 or 10 $\mu\text{g/ml}$ ) [19]. So chrysin NPs could be very successful in alleviating neurodegeneration in epileptic rats.

#### 4.2) Chrysin role in PD

Parkinson's disease is the most common neurodegenerative disorder whose treatment is mainly done by drugs which have serious side effects. Chrysin (5,7 dihydroxy flavone) exhibits several pharmacological activities such as: antioxidant, anti-inflammatory. It may represent a new tool for Parkinson's disease.

Chrysin improved locomotor activity (beam walk, vertical grid test, horizontal grid test) in MPTP induced Parkinson disease mouse model. Chrysin is more effective in a dose of 200 mg/kg as compare to 50 or 100 mg /kg as seen in graph and reduces the time required to cross the beam with period of immobility and in footer slips as compare to MPTP mice. In vertical grid test, chrysin treated mice took less time to climb grid with less immobility period as compare to MPTP mice. But chrysin is more beneficial at 200mg/ kg than 100

or 50mg/ kg. In horizontal grid test, hanging time was seen decreased in chrysin treated mice as compared to MPTP treated mice. Chrysin at 200 and 100 mg/kg shows more significant reduction than 50 mg/kg [20].

It was found that chrysin has the ability to reduce the content of oxidative stress markers in striatum of mice against 6-OHDA. Chrysin increased GSH levels and decrease NOX levels which was found vice versa in 6-OHDA vehicle group 6-OHDA also cause increase in HNE and RS levels in striatum of mice which was further prevented by treatment of chrysin. Chrysin also balances NADPH oxidase and  $NA^+$ ,  $K^+$ -ATPase activities in the 6-OHDA model [21].

Sr. No.	Dose and route of administration	Experimental model	Animal / Cell line	Outcome	Reference
1.	Chrysin (50,100, 200 mg/kg , i.g.)	MPTP Induced Kindling (50, 100, 200 mg/kg)	Male C57BL/6 J mice	Reversed neurochemical deficits, oxidative stress	<a href="#">KRISHNAMOORTHY et.al. 2019</a>
2.	Chrysin (10 mg/kg , i.g.)	6-OHDA Induced kindling ( 0.5 $\mu$ L/min)	Female C57B/6 J mice	Decreased behavioral and cognitive Impairments	<a href="#">DEL FABRO et.al. 2019</a>
3.	Chrysin (50mg/Kg , i.p.)	Rotenone induced kindling (3 mg/kg, i.p.)	Sprague-Dawley rats	Attenuates loss of dopaminergic neurons	<a href="#">AHMED et. al. 2018</a>
4.	chrysin (10mg/kg, per oral, p.o.)	6-OHDA induced Parkinson's Disease (0.5 $\mu$ L/ min )	Male C57B/6 J mice	Neuroprotective effect preserves neurotransmitters such as DA, DOPAC and has antioxidant potential , inhibitory role in neuroinflammation a	<a href="#">GOES ATR et. al 2018</a>

5.	chrysin dissolved in DMSO (50mM stock )	MPTP induced Parkinson's disease	Male C57BL/6 mice	Protection against MPP+ -Induced neurotoxicity and MPTP -induced death. Also attenuated depletion of dopamine and its metabolites	<a href="#">GUO B et.al</a> 2019
6.	Chrysin and PCA	MPTP and 6-OHDA induced Parkinson's Disease	Zebrafish and Rat pheochromocytoma cells (PC12)	Enhanced neuroprotective effects (decreased dopamine neurons) were seen with combination of PCA. Upregulation of nucleolin and HO-1 were also seen.	<a href="#">ZHANG Z et.al.</a> 2015

Del Fabbro L, Rossito Goes A, Jesse CR, de Gomes MG, Cattelan Souza L, Lobo Ladd FV, Lobo Ladd AAB, Nunes Arantes RV, Reis Simionato A, Oliveira MS, Furian AF, Boeira SP. Chrysin protects against behavioral, cognitive and neurochemical alterations in a 6-hydroxydopamine model of Parkinson's disease. *Neurosci Lett.* 2019 Jul 27;706:158-163. doi: 10.1016/j.neulet.2019.05.036. Epub 2019 May 20. PMID: 31121284.

Guo B, Zheng C, Cai W, Cheng J, Wang H, Li H, Sun Y, Cui W, Wang Y, Han Y, Lee SM, Zhang Z. Multifunction of Chrysin in Parkinson's Model: Anti-Neuronal Apoptosis, Neuroprotection via Activation of

MEF2D, and Inhibition of Monoamine Oxidase-B. *J Agric Food Chem.* 2016 Jul 6;64(26):5324-33. doi: 10.1021/acs.jafc.6b01707. Epub 2016 Jun 21. PMID: 27245668.

Goes ATR, Jesse CR, Antunes MS, Lobo Ladd FV, Lobo Ladd AAB, Luchese C, Paroul N, Boeira SP. Protective role of chrysin on 6-hydroxydopamine-induced neurodegeneration a mouse model of Parkinson's disease: Involvement of neuroinflammation and neurotrophins. *Chem Biol Interact.* 2018 Jan 5;279:111-120. doi: 10.1016/j.cbi.2017.10.019. Epub 2017 Oct 18. PMID: 29054324.

Zhang Z, Li G, Szeto SSW, Chong CM, Quan Q, Huang C, Cui W, Guo B, Wang Y, Han Y, Michael Siu KW, Yuen Lee SM, Chu IK. Examining the neuroprotective effects of protocatechuic acid and chrysin on in vitro and in vivo models of Parkinson disease. *Free Radic Biol Med.* 2015 Jul;84:331-343. doi: 10.1016/j.freeradbiomed.2015.02.030. Epub 2015 Mar 11. PMID: 25769424.

### 4.3) Chrysin role in AD

Alzheimer's disease is characterized by loss of neurons in hippocampus and motor impairment. It is disorder of central nervous system. Its pathogenesis and molecular mechanism have not been known. Donepezil and memantine are certain drugs used for this disease but they show certain side effects. Neuroprotective agents (Chrysin belongs to class flavones) are found to be neuroprotective as well as help to improve memory dysfunction. It was found chrysin loaded lipid-core nanoparticles (LNCs) are effective in neurological changes (memory impairment, scape latency, NPSH and RS levels) in a model of AD induced by  $\beta$ -amyloid<sub>1-42</sub>.

This comparison between  $A\beta_{1-42}$  and chrysin suggests that chrysin LNCs (FC, C1-LNC, C5-LNC) are helpful in improving recognition index and memory impairment in ORT against  $A\beta_{1-42}$  induction.  $A\beta_{1-42}$  induction increases scape latency which was seen to be reduced by chrysin LNCs. But C5-LNC is more effective than other chrysin nanoparticles. They also balance the NPSH (contains approx. 90% GSH), BDNF, IL-10 and RS levels [22].

Study also reveals that chrysin Nano-formulation in SLNs helps in increasing the bioavailability and is effective against  $A\beta_{1-42}$  induced oxidative stress. They were effective in controlling biochemical studies, activities of antioxidant enzymes (SOD, CAT, GSH) and balances the levels of vitamin C [23].

### 4.4) Chrysin role in traumatic brain injury

Traumatic brain injury affects different parts of brain and is mainly responsible for neurological condition. It is mainly caused either by accident or sports injury. One must show clinical signs: Decreased consciousness, Memory loss, Difficulty in concentration, loss of vision, Muscle weakness, change in speech. It was seen in

study that chrysin helps in oculomotor dysfunction and memory deficit that are caused by TBI and also improved apoptosis by upregulation of Bcl-2 family proteins expression and decreases the expression of Bax protein [24].

It decreases the effect of neuron inflammation and neuronal apoptosis by the effective dose of 100 mg /kg. TBI anxiety and depression like behavior also seen to be cured by chrysin. It was seen chrysin helps in protection of neurons present in cerebral cortex and hippocampal CA3 areas.

Study shows that chrysin helps detecting apoptotic DNA fragmentation. The associated disabilities such as motor dysfunction and neuronal loss has been improved by chrysin (via increased concentration of SOD, CAT, GPX, GSH and decreased MDA level) [25].

#### 4.5) Role of chrysin in gliomas

Glial cells also called astrocytes helps in supporting of nerve function. The tumor caused by them is glioma which occurs in brain and spinal cord.

Types of glioma include:

- Astrocytomas including astrocytoma and glioblastoma
- Ependymomas, including anaplastic ependymoma, myxopapillary ependymoma
- Oligodendrogliomas, including oligodendroglioma, anaplastic oligodendroglioma and anaplastic oligoastrocytoma

It was shown in study that compound such as CAPE, Chrysin which was found in propolis helps in inhibition of NF- $\kappa$ B. It was seen by the blockage of NF- $\kappa$ B U87MG also decreased in size by 40%. Moreover, complexation of TMZ and EEP (ethanolic extract of propolis) helps in inhibition of glioblastoma.

Chrysin with the increasement of P21<sup>waf1/cip1</sup> protein and by the activation of P38-MAPK levels helps in arrest of G1 phase in C6 glioma cells. Chrysin with combination of pine-needle extracts helps in suppressing MGMT suppression and AKT signaling which shows anti-glioblastoma effects [26].

Chrysin showed greater anti-glioblastoma activity due to its highest inhibition against GBM8901 cells than other compounds (PWE, pinocembrin, tiliroside). Chrysin shows inhibition of growth of GBM8901 cells in range of 25 to 100 $\mu$ M in a time dependent manner. But after 24 h, inhibition was 50% at concentration of 100 $\mu$ M. However, damage of other cell lines (detroit551, NIH3T3, EOC13.31 and rat mixed glial cells) was not seen chrysin as by other compounds. This tells us chrysin shows anti-glioblastoma effect without effecting normal cells. After staining cells with DAPI cytochemical dyes, it was found that chrysin also induces apoptosis in GBM8901 cells after viewing colored fragmented nuclei. Then increasement in apoptotic cells takes place in sub-G1 phase by flow cytometry after get treated with chrysin. Then western blotting technique

is done to examine apoptosis induction by chrysin. The cleavage of caspase 3 and poly (ADP-Ribose) polymerase (PARP) was seen after get treated by chrysin. This shows that chrysin is beneficial at higher concentration (100 $\mu$ M) to reduce proliferation and to induce apoptosis [27].

#### **4.6) Role of chrysin in ischemic brain injury**

Cerebral ischemia is a kind of stroke which takes place due to blockage of artery that supplies blood to brain and results in damage of brain cells. So, it is very important to develop therapeutic agent because after cerebral ischemia many other expressions occur such as: inflammatory cell infiltration, oxygen free radicle reaction which could damage neuronal cells. One of which is flavonoid chrysin. In study, it was found that chrysin could cure neuronal brain cells. It was seen chrysin decreased cerebral infarct size and cerebral edema in ischemic rat.

Firstly, 2,3,5- tri-phenyl tetrazolium chloride (TTC) staining was done to detect infarct size of brain. It was seen cerebral infarct area percentage of rats was increased in I/R model group as compared to SHAM group. After that, decrease in chrysin (10,20mg/kg) or clopidogrel (7.5mg/kg) treatment groups in cerebral infarct area was seen as compared with I/R group. It shows chrysin have the ability to decrease cerebral infarct size.

On the other hand, wet / dry method was used to find water content in the brain to diagnose brain edema. The cerebral edema index was certainly increased in I/R group as compared to sham group. Then chrysin (10,20 mg/ kg) or clopidogrel (7.5 mg/ kg) treatment group decreased the index of cerebral edema as compared to I/R group. It shows chrysin could decrease brain edema index in rats.

Chrysin was able to control ischemic stroke injury by inhibiting inflammation, apoptosis, oxidative stress and neurotoxicity effects. Increased levels of GSH to 235.76% and reduction in TBARS (60.59%), NADPH (36.92%) and XO (34.27) has been shown in I/R group which suggests chrysin is helpful in removing oxidative stress. It also helps in reduction of proinflammatory cytokines TNF- $\alpha$  to 60.31% and IL-6 to 48.72% in the I/R group as shown in and elevation is seen IL-10 levels to 257.19% which tells us about its role in inflammation [28].

#### **4.7) Role of chrysin in multiple sclerosis**

Multiple sclerosis is a common disease of central nervous system which effects myelin sheath which is responsible for protection of neuron, optic nerves and spinal cord. It is an autoimmune disease which affects the body immune system. The animal model used for multiple sclerosis for the pathogenesis and therapeutic interventions is EAE (experimental autoimmune encephalomyelitis).

It has been shown that chrysin depleted mean clinical score, EAE-attenuated increases HDAC activity, GSK-3 $\beta$ , IFN $\gamma$ , IL-17 and TNF levels and decreased HAT3 and HAT4 activity caused by EAE. Histone deacetylase inhibitors (HDACi) are known to be useful in neuroinflammatory disease including MS due to its neuroprotective and immunosuppressive effects. Chrysin helps in blocking HDAC activity in EAE-treated mice. Such inhibition by chrysin shown to have HDAC inhibitor good prophylactic effect in rodent EAE levels [29].

It was found by the measurement of area under curve (AUC) that chrysin EAE treatment results in improvement by 55% in clinical signs (loss of tail tone and motor incoordination) was seen after administration of MOG injection and continued until day 25 of untreated EAE group. Weight loss is also controlled with the help of chrysin. GSK-3B (glycogen synthase kinase 3 Beta) is the main factor responsible for bipolar disorders. It is an enzyme encoded by GSK-3 $\beta$  gene. Chrysin helps in modulation the levels of GSK-3 $\beta$

The study shows that chrysin has very large effects on human DCs. The monocytes in PBMC are specifically eliminated in vitro by chrysin and that chrysin is dose dependent because it inhibits inflammation cytokine production and metabolic activity by PBMCs stimulated by LPS. Chrysin also induce phenotype and function changes in DC. Results suggests that chrysin treated m-DC has the paired potential for reduction of HLA-DR costimulatory molecules and induction of T-cell proliferation. So, inhibitor effect of chrysin on modulation of antigen presentation important in facilitating the pathogenesis of MS and EAE [30]. Chrysin has been found to inhibit NF- $\kappa$ B which further inhibits VCAM expression which has significant role in MS [31].

### **NOVEL DRUG DELIVERY SYSTEM IN IMPROVING CHRYSIN NEUROTHERAPEUTICS**

The pharmacokinetics parameters of chrysin were very poor. It has poor solubility with rapid metabolism and excretion. Therefore, it is very necessary to amplify it so to increase its bioavailability. The methods which could overcome the disadvantages of chrysin are follows: 1) conjugation 2) bottom up manufacturing 3) DDS

It was seen improvement in pharmacological activities of chrysin with the help of chemical conjugation. Conjugation when done between chrysin & indole and barbituric acid, anti-inflammatory activities was enhanced even compared to chrysin itself. Increased anti-cancer effect (via apoptosis-related and antiangiogenics) and antioxidant activities was seen after chrysin-organogermanium conjugation. Chrysin containing selenium exhibits significant cytotoxicity with an IC value 18 times lower than that of chrysin. In addition, the cytotoxic activity of selenium-containing chrysin is higher than that of cisplatin, an anticancer drug. This tells us biological activities of chrysin could be enhanced with the help of conjugation [32]. In bottom-up manufacturing, small molecules are used to develop nanoscale structures. Natural nano-sized foods are a good strategy. Modifications of nanoscale structure could increase solubility by increasing surface-to-volume ratio. Another way to deal with chrysin deficiency is to use drug delivery system. DDS is used to

dissolve a drug or change its properties. It could also be used to assemble encapsulated drugs at targeted sites. Nanoscale carriers coat the drug envelop and accumulate much more in tumor tissue than in normal tissue due to EPR effects. Recently, researchers are working on nanoscale carriers to improve drug concentration at targeted site. DDS as nanoscale carriers is now applicable to cancer therapy and is used to treat infectious diseases. A successful strategy is to select the appropriate carrier for each drug. According to an analysis of literature, DDS uses liposomes, micelles, and nanoparticles as carriers for chrysin. The polymers included in chrysin are manufactured as nanoscale drugs from poly ( $\epsilon$ -caprolactone) (PCL), polylactic acid, glycolic acid (PLGA) and polyethylene glycol (PEG).

METHODS	STRUCTURE	APPLICATION
CONJUGATION	Chrysin-indole-barbituric acid	Anti-inflammatory effect
	Chrysin organogermanium	Anti-oxidant effect
	Chrysin-selenium	Anti-cancer effect
ENCAPSULATION	Liposome	Anti-cancer effect
	Nanoparticles (PLGA-PEG)	Anti-cancer effect
	Nanoparticles (PLGA)	Prevention of <i>S.Typhimurium</i> infection

PLGA is a biodegradable polymer which could be used as a carrier for drug delivery. Due to its small size (50-300nm), it could pass capillaries and shows its action at targeted sites. Chrysin loaded PLGA-PEG used in T47D and MCF7 cell line exerts an inhibitory effect on two breast cancer cell lines more than normal chrysin [33]. Chrysin nanoparticles could be capable to be used in other applications than that of cancer. For example, bee PLGA, chrysin complex and other components are used to prevent *S. Typhimurium* infection [34].

## CONCLUSION AND FUTURE PERSPECTIVE

In coming generation older people will expand over the coming period. This increase in ratio of elder people results in increase of mental disorders. Neuroprotective agents are found to be very successful due to its lower side effects and high efficacy. Some drugs are used for diseases associated with neurons, but they are less effective in correcting the disease and shows various side effects. Many corrective strategies have been developed but each strategy is not very successful. Chrysin (5,7 dihydroxy flavone) found in many vegetables and fruits owns many pharmacological activities: anti-oxidant, anti-inflammatory, anti-cancer, pro-apoptotic properties. Chrysin reduces cognitive dysfunction by diminishing neurotoxicity, neuroinflammation, and oxidative stress. It also play important role in

epilepsy, PD and AD and possess various anti-depressant. However, chrysin is limited by its bioavailability issues which could be increased with the help of bottom-up manufacturing, DDS strategies to attain its therapeutic efficacy. There are many other flavonoids which are useful and have scope in the treatment of certain disorders.

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