Potential Role of Metformin in Central Nervous System with a Focus on its Mechanism of Actions

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Abstract:

Metformin is a common medication for the treatment of type 2 diabetes, which belongs to the biguanide chemical family (T2D). It's interesting that metformin's prescribed use as an anti-diabetic medicine can be expanded due to its therapeutic potential. In this regard, metformin treatment has been shown to have positive benefits on a variety of neurological diseases. In this article, we explore the metformin's positive effects as a neuroprotective agent in various neurological illnesses as well as the various suggested mechanisms of action to better understand how it works on a neurological level.

Recent research has demonstrated that metformin can pass the blood-brain barrier (BBB) and activate particular neurons and neuroglia to exert neurological effects; nevertheless, the precise way in which metformin regulates the central nervous system (CNS) is still unknown. In this review, we compiled the most recent data from preclinical studies that focused on the regulatory function of metformin in the central nervous system (CNS) and discovered that metformin can have potential neuroprotective, neurotrophic, and neurogenesis-stimulating effects. In addition, metformin also has an anti-inflammatory effect by reducing microglial activation and controlling microglial polarisation. These data suggest that the clinical use of metformin for neurological illnesses may have broad pharmacological efficacy and therapeutic insights.

Background: One of the most frequently prescribed medications in the world, metformin is used as the first line of treatment for type 2 diabetes mellitus (T2DM). It has been reported that metformin has beneficial effects on many neurological disorders, including major depressive disorder (MDD), Alzheimer's disease (AD), and Fragile X syndrome (FXS); however, the mechanism underlying metformin in the brain is not fully understood. In addition to its hypoglycemic effects, metformin also can improve cognitive or mood functions in some T2DM patients.

Aim: This review aims to discuss on the various roles of Metformin in the central nervous system and focus on several mechanism of actions by which it controls the CNS.

Methodolology: This review was compiled based on the comprehensive information obtained from research articles and textbooks published on Metformin and its role in CNS, according to the preferred reporting for Systemic Review and Meta-Anaysis (PRISMA). For developing strategic search, various

electronic database systems were used, which include scientific data Scifinder, PubMed, Science direct, ACS publications and Google Scholar.

Result: To better understand the effects of metformin on the brain, we concentrated on and examined the roles of metformin in brain functions and associated neurological illnesses in this review.

Conclusion: We summarised the numerous mechanisms of action and effectiveness of metformin in treating a range of neurological illnesses, and we examined the outcome. Metformin has been discovered to have a wide range of neuroprotective effects, however more testing in various animal models and investigation of its underlying processes are required. We concentrated on the function of metformin in the brain, oxidative stress, neurons, and various neurological illnesses (Epilepsy, Alzeimer, Parkinson etc).

Keywords: Metformin, Mechanism of Action, Neurological Disorders, AMPK, Blood Brain Barrier, Neuroprotectivity.

1. Introduction

One of the most frequently prescribed medications in the world, metformin is used as the first line of treatment for type 2 diabetes mellitus (T2DM). In addition to its hypoglycemic benefits, metformin has been shown to have positive effects on several neurological conditions, including major depressive disorder (MDD), Alzheimer's disease (AD), and fragile X syndrome. In some T2DM patients, it can also improve cognitive or emotional skills (FXS) [22]. Cheap and typically well accepted, metformin has few side effects, little weight gain, and infrequently causes hypoglycemia. The adverse effect profile is generally benign, with short lasting diarrhoea, nausea, and abdominal pain following commencement of metformin treatment often minimised by slowing dose titration [11].

According to pharmacological data, AMP-activated kinase (AMPK) is a crucial cellular target and is connected to the physiological effects of metformin on cells. The metabolic disorders associated with aberrant glucose utilisation, such as prediabetes, polycystic ovary syndrome, and obesity, can also be treated with metformin. Additionally, mounting data suggests that metformin may have positive effects on the non-alcoholic liver, as well as anti-tumor, cardiovascular protection, and anti-inflammatory effects. There is significant evidence that metformin can pass through the blood-brain barrier (BBB) and stimulate particular neurons and neuroglia to elicit a variety of neurophysiological effects. Particularly, age, harm, and stress cause mitochondrial malfunction, which then leads to toxic protein aggregation and, ultimately, to neurodegenerative changes. According to numerous research, metformin therapy can considerably improve behavioural impairments and neuropathological changes associated with Parkinson's and Alzheimer's diseases. Additionally, new research has shown that metformin can stimulate anxiolytic and antidepressant-like reactions by encouraging the brain's uptake of tryptophan. These findings thus demonstrate the existence of unique metformin regulatory mechanisms in the central nervous system (CNS). In this review, we outlined the most recent research on metformin regulation in the central nervous system and elaborated on the drug's therapeutic potential for neurological illnesses [6].

1.1 Pharmacokinetic of Metformin

Metformin enters the portal vein after intestinal absorption and has a 50% to 60% oral bioavailability before accumulating in the liver. Patients who are prescribed metformin get dosages of 1-2g per day (or 20 mg/kg per day), resulting in plasma concentrations of 10 to 40 millimoles of metformin. However, there is a clear disparity in "therapeutic" metformin concentrations in the literature, particularly between in vitro (Table 1) and in vivo (Table 2) trials. The "therapeutic" plasma metformin concentrations reported in earlier in vitro and animal experiments ranged from 1 to 700 M, but many of the studies lacked a citation for their findings. However, there seems to be an almost universal agreement in the literature that metformin plasma concentrations in patients receiving treatment range from 0.1 mg/L to 4 mg/L (from ~1 µM to ~1140 µM).

Several rodent studies have been carried out to establish clinically appropriate dosages and methods of administration based on the reported plasma metformin concentrations in patients with T2D who were given oral metformin [26].

Table 1: Concentrations of Metformin used in In Vitro Studies

Concentration of Metformin in Media	Model	References
Hepatocytes	0.05-5 mM	[1]
Hepatocytes	0.01 mM	[36]
Hepatocytes	0.02-1 mM	[7]
Hepatocytes	0.01-1 mM	[39]
Hepatocytes	0.5 mM	[18]
Hepatocytes	0.25-2 mM	[16]
Hepatocytes	0.02-2 mM	[68]
Hepatocytes	10 mM	[13]
Hepatocytes and H4IIE cells	0.025-10.9 mM	[5]
Hepatocytes and purified enzyme	0.05-0.25 mM	[35]
H4IIE cells	0.5-5 mM	[33]
H4IIE cells	0.05-5 mM	[20]
Isolated mitochondria	10 mM	[44]
Isolated mitochondria	1.25-20 mM	[45]
Isolated mitochondria & astrocytes	0.02-2 mM	[29]
HCT 116 cells	0.25-4 mM	[62]
MCF-7 cells	0.75-5 mM	[65]
15 cancer cell lines	6-200 mM	[64]
S. cerevisiae	0.1-100 mM	[30]

Table 2: Doses of Metformin Used in In Vivo Studies

Model	Route of Administration	Dose	Duration	Observed Plasma/ Peak Metformin	Plasma Glucose Level Decreased or not?	References
Human	Oral	1 g twice/day	Chronic	35 µM (~3 hr)	Not reported	[36]
Human	Oral	1 g	Acute	~20 µM (~3 hr)	Not reported	[36]
Human	Oral	1-3 g/day	2 months	0.1-20 µM*	Not reported	[10]
Human	Oral	1 g twice/day	5 weeks	~10 µM (~6 hr)	Not reported	[56]
Human	Oral	1.7-2.55 g/day	Long-term	~4 µM*	Not reported	[25]
Rat	Oral	3.5 mg/mL	Ad libitum drinking water, 14 days	~15 µM*	Yes	[36]
Rat	Oral	~2.8 mg/mL	Ad libitum drinking water, 4 weeks	Not reported	Not reported	[27]
Rat	Oral	~300 mg/kg	5 days	Not reported	No	[68]
Rat	Intravenous	50 mg/kg	Acute	Not reported	Not reported	[47]
Rat	Intravenous	250 mg/kg	Acute	1300 µM (0.5 hr)	Not reported	[35]
Rat	Intravenous	100 mg/kg	Acute	345 µM (0.5 hr)	Not reported	[35]
Rat	Intravenous	50 mg/kg	Acute	74 µM (0.5 hr)	Yes	[35]
Rat	Intraperitonial	50 mg/kg/day	30 days	Not reported	Yes	[35]
Rat	Intraportal	50 mg/kg	Acute	~130 µM (0.5 hr)	Yes	[36]
Mouse	Oral	250 mg/kg	Acute	~150 µM (1 hr)*	Yes	[22]
Mouse	Oral	1.25 mg/mL	Ad libitum drinking	Not reported	No	[62]

			water, 26 days			
Mouse	Oral	250-500 mg/kg	Acute	Not reported	Yes	[39]
Mouse	Oral	20-50 mg/kg/d	5days	Not reported	Not reported	[16]
Mouse	Oral	50-300 mg/kg	Acute	Not reported	Yes	[16]
Mouse	Intravenous	1.875 mg/[kg-min]	120-minute infusion	~175 µM (end of infusion)*	Not reported	[22]
Mouse	Intravenous	3.75 mg/[kg-min]	120-minute infusion	~350 µM (end of infusion)*	Not reported	[22]
Mouse	Intraperitonial	150-400 mg/kg	Acute	Not reported	Not reported	[18]
High-fat-fed mice	Oral	50 mg/kg/day	Ad libitum drinking water, 6 weeks	Not reported	Not reported	[7]
High-fat-fed mice	Intraperitonial	50 mg/kg/day	6 weeks	Not reported	Yes	[18]
High-fat-fed mice	Intraperitonial	250 mg/kg/day	3 days	Not reported	Yes	[54]

*Time course not available, plasma metformin concentrations displayed as single time point (LaMoia et al., 2021).

Madiraju et al., (2014, 2018) explains that plasma concentrations for non-diabetic patients receiving 1g of metformin orally reach 25 µM within 3 hours after treatment, but peak plasma metformin concentrations for diabetic patients receiving 1g of metformin twice day are around 40 µM.

Timmins et al., (2005) explains another report suggests that peak plasma concentrations ranged from 5 µM to 10 µM in healthy participants treated for 1 week with either 1g of the immediate or extended-release preparations of metformin.

Frid et al., (2010) explains a different research study found that patients with T2D receiving 1 to 3 g of metformin per day for 8 weeks had median plasma concentrations of less than 10 µM.

Madiraju et al., (2014) explains that peak plasma concentrations of metformin following acute intravenous injection of 50, 100, and 250 mg/kg were 74, 345, and 1300 µM, respectively, and liver metformin concentrations reached 100 µM after intravenous treatment of 50 mg/kg of metformin in active rats.

Madiraju et al., (2014), Wang et al., (2018), Chandel et al., (2016) explains that certain studies report, plasma metformin concentrations ranging from 5 µM to 180 µM after oral metformin administration to rodents with unlimited access to metformin-treated drinking water. This variability may be caused by variations in drinking behaviour and/or gastrointestinal metformin absorption.

Wilcock et al., (1994), He et al., (2015) explains that the plasma concentrations of metformin in the portal vein after oral metformin dosing (50 mg/kg) are more significant than systemic plasma concentrations because they are 2- to 3-fold higher than systemic metformin concentrations (10-40 µM), similar to other oral ingested substances.

2. Potential Mechanism of Actions of Metformin
2.1 Mechanism Regulating the Central Nervous System

Treatment with metformin has convergent and exclusive mechanisms for various neurological disorders. Understanding how energy metabolism and neuropathology are related is furthered by looking into the convergent mechanisms of metformin regulation in various neurological disorders. The mechanisms underlying metformin's beneficial effects on mood disorders and neurodegenerative illnesses include the stimulation of BDNF. By reducing circulating branched-chain amino acids, metformin may facilitate serotonergic neurotransmission of the hippocampus, which is known to be strongly associated with depression (BCAAs). Metformin has a well-documented neuroprotective impact in a variety of clinical and neurological disorders. (Figure 1). It is intriguing to note that the regulation of metformin in neurodevelopmental disorders is mediated by the gut microbiota and its impact on microglia. However, the precise cellular and molecular pathways are still unknown [6].
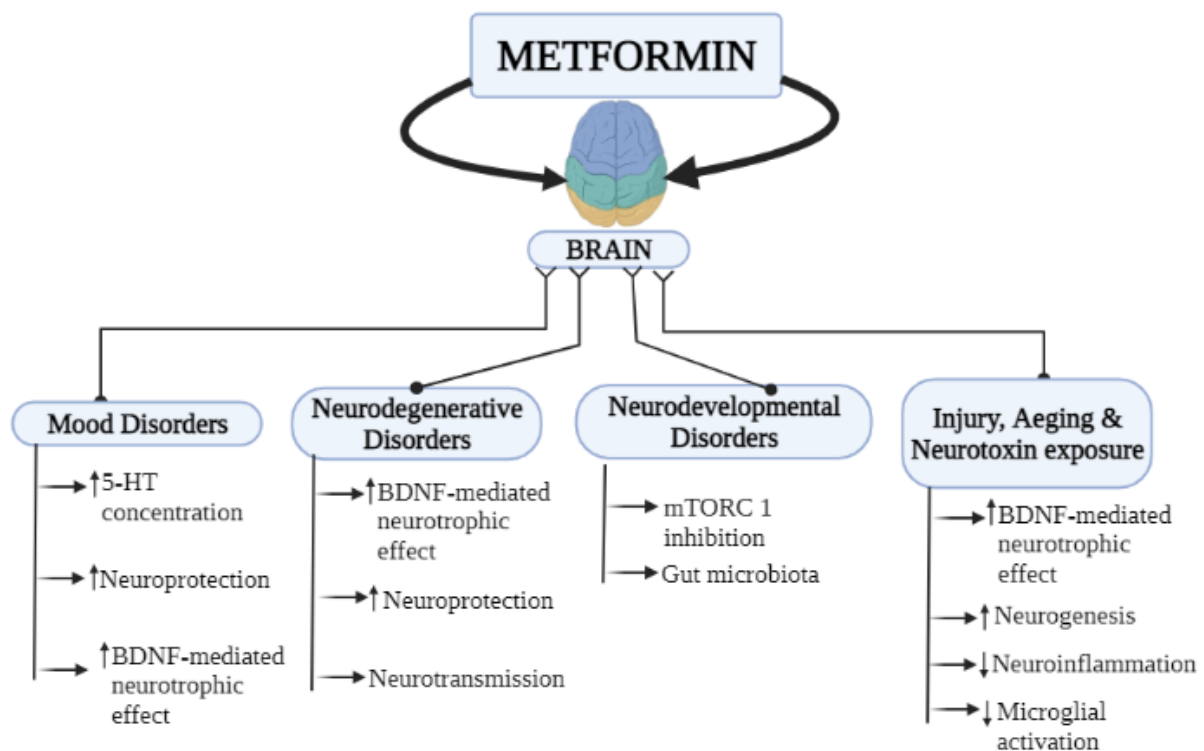

Figure 1: Potential Mechanisms of Metformin Regulation in CNS [6].

2.2 Metformin's Hypoglycemic Effects

Metformin is now commonly acknowledged as an AMPK (AMP-activated protein kinase) agonist (Figure 1). By blocking the mitochondrial respiratory chain complex I, metformin reduces ATP generation while boosting AMP and ADP production, which raises the AMP/ATP ratio. The next step is the inhibition of glucagon-induced cyclic AMP (cAMP) production and activation of AMPK. Through v-ATPase, AMPK may detect low ATP levels, switch cells from anabolic to catabolic states, encourage mitochondrial biogenesis, and control autophagy. A recent study discovered that metformin's new target is presenilin enhancer 2 (PEN2). When metformin is present in small amounts, PEN2 binds to it, inhibits v-ATPase function, and then activates lysosomal AMPK without raising AMP levels. Acetyl CoA carboxylase (ACC) activity decreases as a result of metformin-mediated AMPK activation, which also causes fatty acid oxidation and suppresses the production of lipogenic enzymes. Metformin increases the production of small heterodimer partner (SHP) proteins and reduces hepatic insulin resistance by regulating gluconeogenesis and insulin sensitivity through an AMPK-dependent pathway. Through promoting the phosphorylation of cAMP-response element binding protein (CREB) binding protein, metformin reduces the expression of gluconeogenic genes [28].

Additionally, metformin activates the duodenal AMPK-dependent pathway in rat models of obesity and diabetes, which reduces liver gluconeogenesis and blood glucose levels by inhibiting the mTOR complex 1 (mTORC1) signalling by either directly suppressing Raptor, a crucial component of mTORC1, or by indirectly activating the tuberous sclerosis complex.

Through lysosomal or mitochondrial pathways, metformin activates AMPK. By blocking ACC, AMPK improves insulin sensitivity. Metformin also inhibits the AMP:ATP and NADH:NAD+ ratios through mitochondrial mechanisms or by specifically targeting FBP to reduce glucose in a non-AMPK-dependent manner [28].
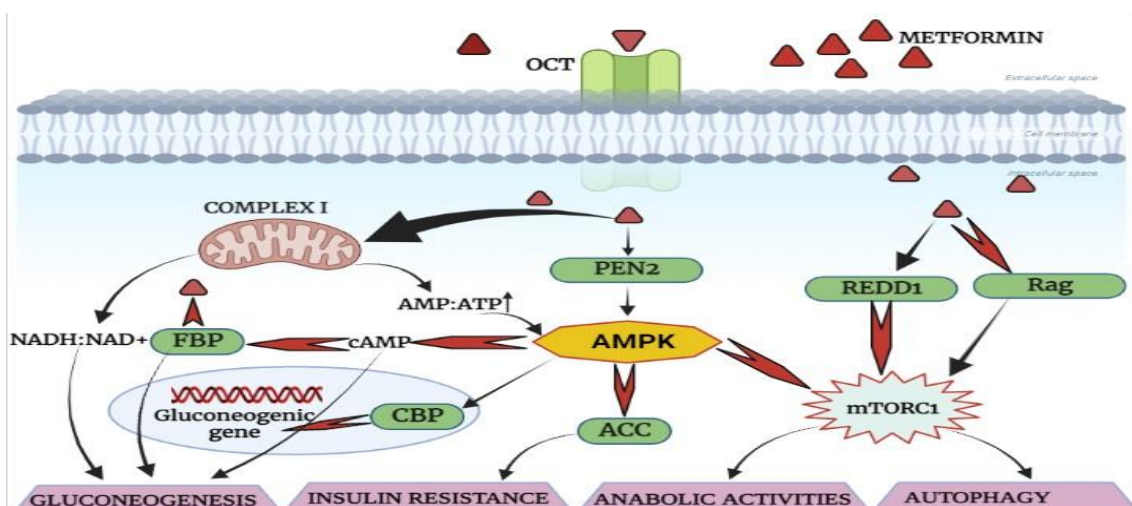

Figure 2: The General Mechanism Underlying the Hypoglycemic Effects of Metformin.

AMPK> 5'-AMP-activated protein kinase;
PEN2> presenilin enhancer 2;
Rag> Rag family of GTPases;

REDD1> regulated in development and DNA damage response 1;

ACC> acetyl CoA carboxylase;

mTORC1> mechanistic target of rapamycin complex 1;

CBP> CREB binding protein;

OCT> organic cation transporters;

FBP> fructose-1,6-bisphosphatase;

AMP> adenosine monophosphate;

ATP> Adenosine triphosphate;

NAD+> the oxidized form of nicotinamide adenine dinucleotide;

NADH> the reduced form of nicotinamide adenine dinucleotide [28].

2.3 Metformins Neuroprotective Properties

When AMPK is activated, insulin resistance improves. On the other hand, persistent insulin resistance can block AMPK and its associated signals. Targeting AMPK can have protective effects on particular energy metabolic states in the peripheral and central nervous systems, according to a number of studies. It has been proposed that the neuroprotective effects of metformin shown in mice suffering from neurological diseases, neurotoxic exposure, and trauma are related to AMPK activation. According to behavioural assay results, metformin therapy reduces the severity of epilepsy or makes it easier for seizures to end, indicating a neuroprotective effect. Numerous studies have shown that metformin activates AMPK to protect neuronal survival from damage. Previous research demonstrated that long-term metformin use inhibits global cerebral ischemia-induced apoptosis through triggering the AMPK/PGC-1 pathway and downstream mitochondrial biogenesis. Forkhead box O-3 (FOXO3), a transcription factor linked to lifespan and a downstream target of AMPK, is one such factor. Metformin has been demonstrated to support the AMPK-FOXO3 pathway to produce an antioxidant stress impact in both in vitro and in vivo studies. In rotenone-induced Parkinson's disease (PD) mice, an 18-day metformin treatment increased the levels of the proteins AMPK-FOXO3 and decreased the levels of VEGF and cleaved caspase 3. Human neural stem cells (hNSCs) exposed to amyloid-β(Aβ) experienced reduced cell viability, which metformin reversed by increasing AMPK and downstream Bcl-2 and cAMP response element-binding protein (CREB) levels, decreasing apoptosis-related caspase 3/9 activity, and decreasing cytosolic cytochrome c levels, for neurodegenerative alterations. Acute metformin intravenous treatment, on the other hand, was observed to reverse brain mitochondrial failure, apoptosis, and Alzheimer's protein aggregation with no appreciable changes in AMPK activities. Similar to this, metformin inhibited MPTP-induced gliosis and reduced TH levels in the Substantia Nigra (SN). These changes persisted in AMPK-KO mice, indicating AMPK plays an elusive role in metformin's neuroprotective effect. Metformin's AMPK-independent energy metabolism-related mechanisms have been extensively studied. These results suggest that the protective effect of metformin may involve other AMPK-independent pathways [28].

2.4 Blood Brain Barrier (BBB)

Disruption of the blood-brain barrier (BBB) is a frequent occurrence in CNS disorders and accidents. In order to facilitate BBB functioning, few medicines have been explored as therapeutic candidates. Takata et al., used rat brain microvascular endothelial cells (RBECs) to investigate whether metformin up-regulates BBB activities. Metformin lowered RBEC permeability to sodium fluorescein and Evans blue

albumin while increasing transendothelial electrical resistance of RBEC monolayers in a concentration- and time-dependent manner. Compound C, an AMP-activated protein kinase (AMPK) inhibitor, prevented these metformin side effects. BBB functions were improved by AMPK stimulation with the AMPK activator AICAR. Their results suggest that metformin activates AMPK to up-regulate BBB activities [55].

The BBB is a selective barrier made up of continuous endothelial cells that line the cerebral microvessels and is encased by mural vascular cells and perivascular astrocyte end-feet. Neuroinflammation, neuronal damage, and synaptic dysfunction all contribute to the breakdown of the BBB and numerous neurodegenerative pathways. The BBB is a complicated, dynamic interface, and treating CNS illnesses requires significant BBB penetration. Oral metformin can easily cross the BBB and build up in the CNS's architecture [28]. It has been established that metformin is an organic cationic transporter (Oct) substrate in the liver and kidneys. Since it has been demonstrated that brain endothelial cells at the BBB express Octs, the theory is that metformin travels across the BBB using Octs [53].

Metformin increases BBB functions in rat brain microvascular endothelial cells by activating AMPK. Treatment with metformin can prevent BBB damage brought on by hypoxia or exposure to vascular endothelial growth factor, as well as reduce the expression of aquaporin-4 protein (AQP4) in vitro. Metformin enhances BBB and neurobehavioral function in rats with a traumatic brain injury (TBI) model by inhibiting TBI-mediated secondary damage through AMPK phosphorylation. By controlling Nrf2 expression, metformin dramatically reduces the effects of smoking on tight junction protein and BBB integrity [28].

2.5 Anti-Inflammation Effect

Inflammation and energy metabolism interact in a complex way. Chronic aberrant metabolic stages are always followed by the emergence of inflammation. Metformin has been demonstrated in animal studies to reduce the neuroinflammation and gliosis seen in mice with HFD or diabetes. Significantly, clinical data showed that metform exert anti-inflammatory effects that are unrelated to participants' levels of diabetes mellitus, suggesting that the antiinflammatory impact of metformin may not be a byproduct of metabolic regulation.

Metformin can prevent the entry of macrophages and the subsequent infiltration of inflammatory cells, which is the first stage of the development of local inflammation, as well as the harm to endothelial cells caused by stress stimuli. The production of filamentous actin stress fibres can be suppressed and P53 inhibition-induced paracellular permeability can be avoided with metformin. Metformin may play a role in preserving the integrity of the epithelium by directly increasing the expression of tight junction proteins, such as claudin 3 (Cldn3) and claudin 5 (Cldn5). In line with that, it has been demonstrated that metformin treatment for seven days after SE exposure can reduce BBB permeability.

There is a lot of evidence that metformin has an anti-inflammatory impact. By preventing NFkb activation, metformin reduces lipopolysaccharide(LPS)-induced neuroinflammation and improves neurological function. The dosage of metformin medication was related to its ability to prevent LPS-induced neuroinflammation. The development of neuroinflammation can be sparked by the activation of

microglia, which function as resident macrophage in the CNS and can detect an inflammatory stimuli. Metformin can block microglial activation and lower levels of pro-inflammatory cytokines, according to a growing body of research. According to the findings of morphological staining tests, metformin can suppress microglial activation, which is mostly exhibited by enhanced ramification and a decrease in the number of active microglia. Along with microglia, astrocytes are another kind of neuroglia that play a role in the development of neuroinflammation. Metformin dramatically reduced the microgliosis and astrogliosis in AD model rat brains. In the cortex and hippocampus of APP/PS1 mice, A plaque load differed in the suppression of inflammation. In the SNI model, pretreatment with metformin also prevents microglia activation by activating AMPK and blocking NFkb signalling. In TBI rats, metformin decreased the phosphorylation of ERK1/2 and p38 MAPK and prevented the translocation of NF-B p65 from the cytoplasm to the nucleus. Similarly, metformin was discovered to block the mTOR/S6K and P65-NF-B signalling pathways and decrease protein Bace1 levels in the hippocampus by activating AMPK to regulate neuroinflammation, which may help to alleviate the neurological abnormalities of APP/PS1 animals. The advanced glycosylation end product (AGE)-treated human neural stem cells' transcript and protein expression levels of increased inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which are indicators of microglia activation, and decreased levels of pro-inflammatory cytokines IL-1, IL-1, IL-2, IL-6, IL-12, and TNF-, were both rescued by metformin, according (hNSCs). By inhibiting mitochondrial ATP and DNA synthesis, metformin can block the activation of the NLRP3 inflammasome and the production and secretion of pro-inflammatory proteins. The proinflammatory mediators IL-6, IL-1, and CXCL1/2 are also inhibited by metformin in macrophages, but not M1/M2 differentiation or activation. The anti-inflammatory effect of metformin is attributed to compensatory mechanisms linked to Nrf2 control in oxidative stress. In mice treated with HFD, it was discovered that extended metformin administration decreased nrf2 transcription. Significantly, it has been demonstrated that metformin increased dopaminergic damage after MPTP injection, while still preventing microglia activation and ROS production by blocking the NADPH oxidase enzyme and MAPKs. These results suggest that metformin's beneficial effect on neurological dysfunction may be largely explained by its ability to reduce neuroinflammation. The contradictory impact of metformin on neuropathogenesis may be explained by the dual function neuroinflammation played in various types or stages of neurological disorders [6].

3. Rationale Underlying the Selection of Metformin in Treating Different Neurological Disorders

A rising interest in the drug's potential therapeutic benefits for conditions of the central nervous system. It is now known that many neurodegenerative diseases share pathological mechanisms of neuronal and support cell damage that parallel stress pathways in other diseases, such as type 1 diabetes (T1D) and type 2 diabetes (T2D), despite the fact that most neurodegenerative diseases are heterogeneous in nature. The mTOR signalling pathway is one example of a pathogenic mechanism present in a variety of illnesses. Notably, metformin suppressed rapamycin complex 1 (mTORC1), the mTOR signalling pathway's molecular target, through both AMPK-dependent and AMPK-independent pathways. This supports the hypothesis that, due to common molecular abnormalities shared with T2D, metformin has the potential to affect a variety of neuropsychiatric and neurodegenerative illnesses [11].

Since metformin has already received approval for usage in humans, it is an intriguing medicine since it has the potential to affect neuronal lifespan pathways. However, the absence of effective ageing models

that can be applied in the laboratory has slowed back research on human ageing in general [38]. Direct reprogramming techniques have made it possible to preserve ageing signs in reprogrammed neurons, however this approach may not be practical for all research teams, and it may take some time for the technologies to become established in less-experienced labs. The demand for fresh, straightforward, and reasonably priced approaches to study how human cells age is still quite strong [23].

Regardless of cell type, findings from human and animal research have demonstrated that insulin function dysregulation plays a role in ageing and the emergence of neurodegenerative disorders [69]. Diabetes and insulin resistance are now more widely acknowledged as factors in the development of disease, particularly in the area of dementias [42]. The justification for using metformin is that it may reduce the ageing process by affecting insulin signalling and mitochondrial metabolism [41]. Delaying disease may increase quality of life in old age, therefore slowing the ageing process will be advantageous [48,57].

4. The different roles of Metformin in CNS are

4.1 Metformin Reduces Oxidative Stress

There is strong evidence that metformin has antioxidant properties. Some of these are related to the suppression of mitochondrial complex I, which lessens the generation of reactive oxygen species (ROS) by the OXPHOS respiratory chain. In addition, metformin has additional effects on the AMPK pathway, including the following:

(i)   Reducing the activity of NADPH oxidase, a major source of cellular ROS;

(ii)   Increasing PGC1alpha transcription factor activity to increase mitochondrial biogenesis;

(iii)   Lowering ROS concentrations by enhancing the production of antioxidant enzymes such thioredoxin via the AMPK-FOXO3 pathway and

(iv)   Altering sirtuin 3 (SIRT3) deacetylase expression, whose activity encourages antioxidant actions in cells [52].

4.2 The Impact of Metformin on Brain Glucose Metabolism

Metformin affects glucose metabolism in a variety of organs, including the brain, in addition to hepatocytes. It is known that the metabolic profiles of glucose differ between neurons and astrocytes. Due to the peculiarities of the various glycolytic enzymes present in neurons, the metabolism of these cells depends more on mitochondrial oxidative phosphorylation (OXPHOS). Because of this, neurons have a fully active form of pyruvate kinase (Pkm1), and the resultant pyruvate is quickly converted into acetyl-CoA by a fully active type of mitochondrial pyruvate dehydrogenase (Pdh). The amount of lactate produced by neurons is extremely minimal because they express a lactate dehydrogenase isoform (Ldh1) that functions better by converting lactate into pyruvate. Additionally, they express the monocarboxylate transporter 2 (MCT2), which is totally functional in absorbing lactate from the environment. Finally, neurons are ready to take up lactate and transform it into pyruvate, which will enter the mitochondria to produce energy. However, the glucose metabolism of astrocytes is more glycolytic. Due to the low activity of their mitochondrial pyruvate dehydrogenase (Pdh), which is inhibited by an active pyruvate dehydrogenase kinase 4, they have diminished OXPHOS (Pdk4). The monocarboxylate transporters MCT1/4 export lactate from astrocytes, which is produced by the expression of an isoform of lactate dehydrogenase (Ldh5) that is completely active in the process of converting pyruvate to lactate. As a

result, astrocytes' glucose metabolism is built to result in lactate production and lactate export to the environment. With all of this information in mind, we hypothesise that metformin would reduce gluconeogenesis in neurons and astrocytes at the central nervous system (CNS) by inhibiting mitochondrial Gpd2 and activating AMPK, which would result in a drop in DHAP and pyruvate levels, similar to what happened in hepatocytes (see above); in addition, this would result in an increase in the glycolytic flux (use of Glu-6P). As a result, astrocytes and neurons would produce more lactate and OXPHOS, respectively. Due to metformin-mediated activation of AMPK, which activated Pfkfb3 and increased Pfk1 activity, glycolysis would also rise in astrocytes. The intake of glucose and the breakdown of glycogen would both be expedited to meet the increased demand for Glu-6P. As glycogen synthase would be rendered inactive by metformin-mediated activation of AMPK, we anticipate that glycogen levels will drop following metformin administration. This could explain why metformin treatment stopped the production of polyglucosan in mice models of Lafora illness [52].

4.3 Metformin's Role in Neuro Inflammation

Neuroinflammation is initially neuroprotective after brain injury, but when it becomes chronic or excessive, it eventually results in damage. It is now widely acknowledged that ongoing brain inflammation encourages neuronal hyperexcitability and seizures, and that dysregulation in the glial cells' immune-inflammatory function frequently predisposes people to seizures or plays a role in their development. A cascade of inflammatory mediators is subsequently released when acute convulsions upregulate the production of pro-inflammatory cytokines in microglia and astrocytes. As a result, inflammatory mediators and epileptic convulsions create a positive feedback loop that reinforces one another. However, only specific anti-inflammatory compounds, whose selection has been made after a thorough understanding of the main related inflammatory pathways, should be used with each specific type of epilepsy, as the use of general anti-inflammatory drugs is not advised due to their detrimental performance in long-term treatments. The majority of neurological illnesses exhibit NF-kB activation, which is a characteristic of neuroinflammation. NF-kB is a nuclear factor kappa-light-chain-enhancer of activated B cells. Inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2), cytokines and chemokines, are examples of pro-inflammatory mediators that are expressed as a result of the activation of NF-kB via the Toll-like receptor 4 (TLR4) signalling pathway (iNOS) through myeloid differentiation primary response 88 (MyD88) and tumour necrosis receptor-associated factor 6 (TRAF6). Since metformin inhibits the signalling of NF-kB as well as the expression of pro-inflammatory cytokines (interleukin 1-beta (IL-1beta), interleukin-6 (IL-6), tumour necrosis factor alpha (TNFalpha), C-C motif chemokine ligand 2 (CCL2), etc.) in various cell types, it has been reported that AMPK activation by metformin reduces general inflammatory conditions. A similar reduction in the synthesis of IL-1beta, IL-6, CCL2, and CXCL2 also reducing activated microglia; neutrophil infiltration; is seen following traumatic brain injury due to berberine's activation of AMPK. Both times, AMPK stopped the TLR4/NF-kB signalling pathway from being activated. By reducing LPS-induced, TLR4-mediated NF-kB activation, AMPK also reduces the lipopolysaccharide (LPS)-induced expression of proinflammatory cytokines (TNF-alpha, IL-1beta, and IL-6) in the body. Similar to this, in AGE-treated human neural stem cells, AMPK stopped the advanced glycation end-product (AGE)-mediated signalling cascade, which leads to a rise in NF-kB expression and a decrease in iNOS and COX2 levels (hNSCs). The suppression of LPS-induced activation of the PI3-kinase/RAC-alpha serine/threonine-protein kinase (Akt) pathway by AMPK was likewise linked to the anti-inflammatory effects of AMPK. The

nucleotide-binding oligomerization domain and leucine-rich repeat and pyrin domain 3 (NLRP3) inflammasomes are inhibited when NF-kB levels are downregulated, whereas caspase1 is activated less and less IL-1beta is produced less. These pathways are particularly crucial in astrocytes, where AMPK prevents inflammation caused by high ER stress and hyperglycemia, and microglia, where AMPK prevents the production of pro-inflammatory markers, reducing neuroinflammation.

Since neuroinflammation has been linked to neurological conditions like epilepsy, Parkinson's, Alzheimer's, and Huntington's illnesses, metformin may benefit these conditions [52].

4.4 Neuroprotective Effect of Metformin in Different Neurological Disorders

The potential use of metformin in disorders of the central nervous system is gaining more and more attention (CNS). Although the aetiology of the majority of neurological disorders varies, they all share fundamental pathogenic mechanisms that are changed in the corresponding disease. The mechanistic target of rapamycin (mTOR) kinase and AMPK pathways are two examples of this. As previously mentioned, the primary regulator of energy homeostasis is AMPK. It is triggered when there is a lack of energy, and by triggering catabolic pathways while suppressing anabolic pathways, it restores the equilibrium of the body's energy systems. The mTOR pathway, in contrast, is activated in high-energy situations and works by triggering anabolic and suppressing catabolic pathways. The AMPK and mTOR pathways are intertwined, and activation of the AMPK pathway inhibits the mTOR system by either directly inactivating mTOR complex components (such as Raptor and the Tuberous Sclerosis Complex 2 (TSC2)) or by flipping the mTOR impact on common substrates (e.g., ULK1 in the autophagy process). Metformin specifically stimulates AMPK signalling and blocks mTOR signalling through AMPK-dependent and AMPK-independent mechanisms. The activity of metformin as a neuroprotector drug in various neurological illnesses will be briefly discussed in the sections that follows:

4.4.1   Parkinson's Disease

The second most common neurodegenerative ailment is Parkinson's disease. The death of dopaminergic neurons has been linked to elevated levels of reactive oxygen species (ROS) and increased oxidative stress, which have been regarded a consequence of mitochondrial malfunction in the course of disease. Metformin therapy lowers oxidative stress and enhances the production of antioxidant enzymes including catalase and superoxide dismutase. Both the AMPK pathway and AMPK-dependent pathways, such as the activation of the brain-derived neurotrophic factor (BDNF) signalling pathway, were used by metformin to produce this effect. Metformin suppresses alpha-synuclein aggregation, avoids mitochondrial malfunction, reduces oxidative stress, promotes autophagy through activation of AMPK, and decreases neurodegeneration and neuroinflammation in animal models of Parkinson's disease [52].

4.4.2   Alzheimer's Disease

The neurodegenerative ailment with the highest prevalence is Alzheimer's disease (AD). Progressive memory loss and compromised cognitive function are its hallmarks. Clinical studies show that metformin has a protective effect against cognitive deterioration and Alzheimer's disease. Actually, compared to other T2D patients getting alternative treatments, metformin-treated patients had a lower risk of cognitive decline and a lower risk of acquiring Alzheimer's disease. Additionally, AMPK

activation has been shown to have neuroprotective effects in various Alzheimer's disease-caused mice models [52].

4.4.3 Epilepsy

A neurological illness known as epilepsy is characterised by a propensity to have epileptic seizures and the related cognitive, psychological, and social effects of this condition. Around 65 million individuals worldwide suffer from epilepsy, which is brought on by acquired brain injuries (such as those following a stroke or traumatic brain damage), infectious diseases, autoimmune diseases, and genetic abnormalities. Epilepsy affects 1% of the world's population as a whole. More than 500 genes have been linked to epilepsy thus far. Anti-seizure medications are the first-line treatment for epilepsy (ASDs). The neuron-centric hypothesis, which holds that an imbalance of excitatory and inhibitory currents accounts for the majority of epileptic seizures, served as the foundation for the development of ASDs. Nevertheless, despite the abundance of ASDs, about one-third of patients are unable to manage their seizures or quickly develop a resistance to the drugs' effects. Therefore, it is imperative to create cutting-edge antiepileptic therapy plans in order to accelerate illness progression and reduce its negative effects. Different treatment approaches have been developed to block the mTOR pathway since various kinds of epilepsy are associated with the upregulation of this system. The middle cerebral artery occlusion (MCAO) model of cerebral stroke shows improved motor deficits when mTOR signalling is inhibited because microglia produce fewer pro-inflammatory cytokines and chemokines (IL-1beta, TNF alpha, CCL2, iNOS, etc.) as a result. As previously mentioned, AMPK is a master regulator of energy homeostasis. It is activated in situations of energy scarcity and restores energy balance by triggering catabolic and blocking anabolic pathways. The mTOR pathway, in contrast, is activated in high-energy situations and works by triggering anabolic and suppressing catabolic pathways. Similar to other peripheral tissues, the CNS activates these pathways. It's interesting to note that the AMPK and mTOR pathways are related, and that activating the AMPK route inhibits the mTOR system (see above). Metformin specifically activates AMPK, which suppresses mTOR signalling and enhances seizure control in models of mTOR overactivation [52].

4.4.4 Multiple Sclerosis

A persistent autoimmune condition called multiple sclerosis results in demyelination and the death of neuronal cells in the central nervous system. The foundation of current therapeutic strategies is the control of autoimmune assaults and the maintenance of oligodendrocyte function. In patients with multiple sclerosis, metformin reduces oxidative stress and restores mitochondrial function. Additionally, in an experimental model of autoimmune encephalomyelitis, metformin was able to increase the expression of genes related to oligodendrocyte protection and the recovery of central nervous system functions in an AMPK-dependent way [52].

4.4.5 Huntington's Disease

A faulty huntingtin gene is the cause of the autosomal, dominant hereditary illness known as Huntington's disease (Htt). Long poly-Q tracks on the mutant huntingtin protein overburden the ubiquitin proteasomal degradation machinery and cause it to combine with other proteins, depleting essential components for maintaining neuronal homeostasis and causing neuronal degeneration. According to reports, metformin treatment has been shown to decrease the amount of huntingtin

aggregates, most likely as a result of activating autophagy, which happens when the AMPK pathway is activated. In mice models of Huntington's disease, a decrease in huntingtin aggregates is associated with an increase in cognitive and behavioural function [52].

4.4.6 Fragile X Syndrome (FXS)

Chronic improvements in irritability, social responsiveness, hyperactivity, and social avoidance were seen in seven FXS patients who were given metformin. In a drosophila FXS model, metformin restored impairments in long-term memory and enhanced olfactory learning. Metformin restores long-term depression, altered spine morphology, and enhanced grooming and social behaviour impairments in the Fmr1 knockout (KO) mice model of FXS. It also preferentially normalises ERK signalling and the production of matrix metalloproteinase-9 (MMP-9) [28].

4.4.7 Major Depressive Disorder (MDD)

MDD is a diverse condition whose mechanism is poorly understood and for which there are no reliable biomarkers. Compared to the control group, teenagers taking additional metformin who had severe mental illness (schizophrenia spectrum disorder, bipolar spectrum disorder, or psychotic depression) reported considerably fewer incidents of aggression and impulsivity. The inclusion of metformin did not significantly vary from the control group, but it did help prevent the weight increase that children who take antipsychotics have and had no unfavourable side effects. In contrast to pioglitazone, prolonged usage of metformin and combination therapy was linked to a decreased incidence rate of depression in a nationwide population-based investigation. The Hamilton Depression Rating Scale (HDRS) score of MDD patients who take metformin as an addition to fluoxetine is higher, and the response and remission rates are higher compared to the placebo group in a double-blind, placebo-controlled experiment. In a clinical research, all participants took fluoxetine and had T2DM and post-stroke depression. The metformin subgroup experienced a small reduction in depressive symptoms compared to baseline, whereas pioglitazone had a more notable antidepressant effect. Pioglitazone outperformed metformin in a six-week double-blind study of 50 individuals with polycystic ovarian syndrome (PCOS) and MDD in terms of lowering HDRS scores. Metformin demonstrated a slight but statistically significant improvement in the Beck Depression Inventory and the Quality of Well-Being Scale in a randomised controlled trial in obese adults (BDI). In a clinical research, female patients with MDD who were diabetic or prediabetic received chronic metformin treatment, which dramatically improved cognitive function. The BDI score of the PCOS participants marginally increased after the 12-week metformin intervention. Metformin is helpful for reducing depressed symptoms, according to the majority of clinical trials on depression-related illnesses. Metformin reduced depressive-like symptoms brought on by outside stimuli in animal studies. Metformin reduces depressive-like behaviour, corrects synaptic abnormalities brought on by chronic social defeat stress (CSDS), and increases the expression of brain-derived neurotrophic factor (BDNF) in the chronic social defeat stress (CSDS)-induced depression mice model. Metformin treatment reduced depressive-like behaviours and fixed aberrant glutamatergic transmission in LPS-treated mice. Metformin increased excitability and neurotransmission of 5-hydroxytryptamine (5-HT) neurons in mice fed a high-fat diet (HFD), while inhibiting HFD-induced anxiety by lowering circulating branched-chain amino acids. Metformin has antidepressant benefits because it can reduce depressive-like behaviours in corticosterone-induced metabolically disturbed rats [28].

4.4.8 Metformin's role in Lafora Disease

The accumulation of Lafora bodies (LBs), also known as insoluble glycogen-like inclusions (polyglucosans), in the brain and other tissues, is a hallmark of Lafora disease, an extremely rare form of autosomal, recessive progressive myoclonic epilepsy (PME). LBs can be detected using periodic acid Schiff (PAS) staining. Lafora is a neurodegenerative condition that is also referred to as a glycogen storage condition. The molecular foundation of the disorder has been studied, as well as the quest for effective treatments, using a variety of genetically altered animal models that display a wide range of symptoms present in patients with the disease. Metformin is one of the medications that successfully reduced their symptoms, and as we will see below, it is already utilised in clinical settings [52].

5. Lafora Disease Clinical Features

Lafora illness is a deadly neurological condition that typically develops in kids between the ages of 10 and 15 who seem to be developing their brains normally. The earliest signs include epileptic seizures, myoclonus, and/or cognitive changes that make it difficult to concentrate in class. The language and intellectual issues start off very quickly after the cognitive and neurological degeneration, and they continue to become worse until the patients experience severe dementia, ataxia, dysarthria, amaurosis, and respiratory failure. The condition has no known cure; anticonvulsant medications are used to treat seizures only, but patients quickly build up a tolerance to them, and myoclonus develops into a permanent condition. Death frequently follows status epilepticus or aspiration pneumonitis within the first five to ten years of diagnosis.

Mutations in EPM2A (epilepsy of progressive myoclonus type 2 gene A) or NHLRC1/ EPM2B (NHL repeat-containing protein 1/epilepsy of progressive myoclonus type 2 gene B), encoding the laforin or malin proteins, respectively, have been described as causes of the disease. About 60% of patients with Lafora illness have mutations in the EPM2A gene, while 35% have mutations in the EPM2B gene. Those with EPM2B gene mutations appear to have a milder phenotype than patients with EPM2A gene mutations. Additionally, it has been noted that major phenotypic variances are linked to mutations in the same gene and even the same mutation. Therefore, it has been proposed that the age of onset and severity of the disease may be influenced by genetic or epigenetic modifying variables. In addition to other physiological mechanisms, laforin and malin collaborate to control glycogen synthesis, maintain proteostasis, maintain the homeostasis of glucose transporters, and respond to oxidative stress [52].

6. Metformin Therapy for Neurological Diseases

The efficacy and safety of metformin as described in prior hypoglycemia studies have sparked interest in research into its therapeutic potential in neurological diseases. According to a number of clinical studies, using metformin in combination with psychiatric medications can help prevent side effects including weight gain or obesity. Additionally, it was discovered that metformin can help those with non-dementia vascular cognitive impairment (NDVCI). Particularly, metformin can pass across the BBB and then directly influence neurons, suggesting that it may have a part in neurological illnesses. As a result, we outlined the most recent animal research on metformin's impact on neurological illnesses, particularly mood, neurodegenerative, and neurodevelopmental disorders. One of the most prevalent behavioural signs of neurologic dysfunction is cognitive impairment, which is caused by complex interplay between

environmental and hereditary influences. Metformin can enhance pathogenic and cognitive traits in animal models of neurodegenerative illness, mood disorders, and neurodevelopmental disorders, as shown in Table 1. Metformin treatment was observed to improve the cognitive dysfunction and neuropathic pain that were present in diabetes animals. Metformin's reduction of neuropathic pain was also noted in nerve injury (SNI) animal studies. Further metformin can increase the improvement of depressive and schizophrenic-like behaviours brought on by antipsychotic or antidepressant medication. Additionally, it was discovered that metformin reduced anxiety and depressive-like behaviours in mice given with a high-fat diet (HFD) and APOE4 carriers. It was recently discovered that long-term metformin use reduces the severity of seizures brought on by pentylenetetrazole (PTZ) or kainic acid. Nevertheless, conflicting research suggests that metformin pretreatment had no appreciable impact on the cognitive abnormalities brought on by HFD. Furthermore, it was discovered that metformin enhanced the phosphorylation of tau and decreased the number of NeuN- and PSD95-positive cells in ApoE-/- mice, and that the mechanisms may involve increased production of lipogenic genes. Metformin was discovered to worsen males' memory dysfunction while protecting females in AD models via activating AMPK [6].

Table 3: Effect of MetforminTreatment on Neurological Disorders

Neurological Disorders	Animal Model used	Related Disease	Mechanism of Action	Final Report	Reference
Neurodegenerative	Parkinson	MPTP model	Due to BDNF - mediated neurotrophic effect	Enhancing the levels of BDNF and α-synuclein phosphorylation.	[24]
		MPTP model		Antioxidant activity and BDNF levels are increased along with enhanced muscle performance and locomotor activities.	[46]
		MPTP model	Due to Neuroprotection but independent of AMPK activation	Gliosis prevention occurs; TH neuronal loss is prevented.	[4]
		MPTP model	Due to Neuroprotection	Autophagy stimulation and mitochondrial ROS removal.	[34]
		Clk (+/-)	Due to AMPK-dependent	Dopaminergic neuronal death is	[67]

		Mutant mouse model	neuroprotection	decreased and AMPK/mTORC1-mediated autophagy is activated.	
		Rotenone model	Due to AMPK-dependent neuroprotection	Decreased neuronal death; expansion of TH neurons; AMPK-FOXO3 signalling pathway activation.	[12]
		Rotenone model	Due to Antiinflammation; BDNF-mediated neurotrophic effect; Neurogenesis.	BDNF production and neurogenesis are increased; iNOS and NF-κB, IL-1β level inhibition; depressive-like symptoms are inhibited.	[37]
		6-OHDA model	Due to effect of Anti-inflammation and BDNF - mediated neurotrophic effect.	Reactive astrocytes' expression of aging-induced genes being suppressed; AMPK/BDNF signalling activation.	[49]
		6-OHDA model	Due to Neuroprotection	The enhanced activity of GSK3β is controlled by the overexpression of ERK1/2 and D1R.	[50]
		6-OHDA model	Due to Neuroprotection	GSK3β stabilization of GSK3β occurs; D1R, mTORC and ERK1/2 are increased in the striatum.	[50]
	Alzeimer	SAMP8 Mouse model	Due to Neuroprotection	Treatment with 200 mg/kg, PKC, PKC, PKCζ, PKCα	[15]

				and pGSK-3ββser9 increases and Aβ, and pTau decreases.	
		(P301S) transgenic mouse model	-----	In the cortex and hippocampus, reduction of the phosphorylation of tau occurs due to the activation of PP2A and AMPK.	[3]
		APP/PS1 mouse model	Due to Neurotransmission	Bring back the expression of LTP, spatial memory and surface GluA1 trafficking.	[60]
		APP/PS1 mouse model	➢ Due to Neuroprotection ➢ Due to AMPK-dependent neuroprotection	➢ controlling the AMPK/P65 NF-B signalling pathway as well as the AMPK/mTOR/ S6K/Bace1 pathway ➢ enhancing and turning on BACE1 transcription.	➢ [43] ➢ [9]
		APP/PS1 mouse model	Due to Cellular autophagy mediated neuroprotection	Autophagy is mediated by TAK1-IKK/Hsc70 signalling.	[66]
	Huntington	Hdh 150 knock-in mouse model	Due to Neurotransmission	Reactivate the hyperactive neurons and improved synchrony and reduces the abnormal huntingtin protein load.	[2]
		zQ 175 mouse	Due to BDNF-mediated	Increase in BDNF levels; nuclear	[51]

		model	neurotrophic effect	aggregate count reduction.	
		zQ 175 mouse model	Due to Microglia activity; BDNF-mediated neurotrophic effect.	Microglial activation being inhibited; BDNF levels being increased, and AMPK activation.	[51]
Neurodevelopment al disorder	Autism	BTBR T+Itpr3 tf/J mouse model	------	Reversal of repeated grooming, marble burying, and social approach impairments	[61]
		Bmal 1 KO mouse model	Due to Neurotransmiss ion; mTORC1 inhibition.	Impairments in the restoration of the PCs firing; mTORC1 suppression; Immediate relief of the main cognitive dysfunction of ASD.	[31]
	Fragile X syndrome	Fmr1-/y mouse model	Due to prevention of ERK and mTOR signalling hyperactivation	ERK signalling, eIF4E phosphorylation, and MMP-9 expression are returned to normal; key behavioural abnormalities are reduced.	[19]
		Drosophila Fragile X model	-------	Restoration of memory impairments; Insulin signalling inhibition.	[40]
Mood disorder	Depression	CUS model	Due to Neuroprotectio n	Reverse the decreased p-GLP1 and ERK and the elevated BAX.	[32]
		SDS model	Due to BDNF - mediated	Positive impact on the neurite	[14]

			neurotrophic effect	plasticity of CA1 pyramidal neurons due to activation of the AMPK-CREB-BDNF pathway	
		RS model	Due to BDNF - mediated neurotrophic effect	The AMPK/Tet2/BDNF pathway is activated	[59]

CUS: Chronic unpredictable stress;

SDS: Social defeat stress;

RS: Restraint stress;

LTP: Long-term potentiation;

BDNF: Brain-derived neurotrophic factor;

D1R: Dopamine D1 receptor;

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;

6-OHDA:6-Hydroxydopamine;

ROS: Reactive oxygen species;

S6K: S6 kinase;

TH: Tyrosine hydroxylase;

GluA1: glutamate receptor 1;

HD: Huntington disease;

MMP-9: Matrix metalloproteinase-9;

ERK: Extracellular-signal regulated kinase;

ASD: Autism spectrum disorder;

KO: Knockout;

NA: Not applicable;

IL-1β: Interleukin 1β;

iNOS: inducible nitric oxide synthase;

GSK3β: Glycogen synthase kinase 3β.

7. Conclusion

We concluded by summarising the clinical use of metformin and its effectiveness in treating various neurological diseases. Metformin has been discovered to have a wide range of neuroprotective effects, however more testing in various animal models and investigation of its underlying processes are required. This review concentrated on the function of metformin in synaptic, astrocyte, and neuronal transmission. Finally, more research is required to determine the precise mechanism underlying the neuroprotective properties of metformin as well as the many causes of its adverse effects. Due to its low incidence of clinical side effects, metformin may prove to be a promising treatment for avoiding neurological problems in the future.

On the other hand, recent evidence reported that metformin alleviates the core behavioral deficits in neurodevelopmental diseases. Considering that the etiology of neurodevelopmental diseases has strong basis on genetic background, metformin regulation in these diseases might have separated mechanisms from that in metabolism dependent diseases. The specific mechanisms still warrant further investigations.

8. Conflict of Interest

The authors report no conflict of interest.

9. Acknowledgement

10. Author Contributions

Kangkana Bora (Corresponding Author): Wrote the article, designed the references as per the journal's requirements. Professor Rajana James comprehended the idea, provided the guidance and resources and reviewed the article as per the journal's requirement.
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