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Review Article

Insights into the neuroprotective properties of Biochanin-A

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ABSTRACT

The global morbidity and mortality caused by neurological disorders are significant. Neurodegenerative disorders are anticipated to rise as the population ages because they typically manifest in mid-to-late life. The World Health Organization predicts that by 2050 two billion individuals would be 60 or older. So there is an emerging need for neuroprotective agents derived from natural sources with favorable efficacy and high safety profile. One of these natural agents is biochanin A(BIO-A), an isoflavone belonging to phytoestrogens, mainly found in red clover, soy, and chickpea, commercially available tablets. It has a wide range of pharmacological effects, such as antioxidant, anti-inflammatory, anti-apoptotic, antimicrobial, Estrogen-like, glucose and lipid metabolism modulatory, anti-cancer, and neuroprotective effects. BIO-A was proven to be promising when investigated in multiple models of neurological diseases such as Alzheimer's disease and Parkinson's disease, multiple sclerosis, stroke, brain injury, depression, anxiety, and glioblastoma. This review focuses on the possible molecular mechanisms responsible for the neuroprotective effects of BIO-A in various neurological disorders.

Keywords: *Biochanin A; neuroprotective; anti-inflammatory; Alzheimer's; Parkinson's diseases; glioblastoma*.

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1. INTRODUCTION

Worldwide, neurological diseases are the second major cause of death and the main cause of disability. The increased occurrence of neurological disorders has adversely affected people's quality of life, causing a significant impact on society and the economy with a growing burden of death and disability. In 2019, region-wide in USA, neurological disorders accounted for 533,172 deaths, 213,129 in men, and 320,043 in women, and 8.2 million years lived with disability (YLDs), 3.1 million Years of life lost (YLLs) in men, and 5.1 million YLLs in women. Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), brain tumor, stroke, and traumatic brain injury (TBI) are the most prevalent neurodegenerative diseases accounting for significant disability worldwide **[1, 2]**. Because of the increased prevalence of injury-related and non-communicable neurological diseases, research

has concentrated on the design of appropriate treatment plans **[3]**. The majority of the currently available pharmacotherapeutic approaches just alleviate the symptoms. Furthermore, drugs' side effects often deteriorate the quality of life of patients. Thus, the bulk of research in the last decade has focuses on identifying appropriate substitutes with higher safety. On this point, research concentrate on medicinal plants with broad pharmacological activities and favorable safety profile **[3, 4]**. Of these safe classes of compounds are the Phytoestrogens which are polyphenolic and non-steroid compounds that are naturally present in a huge number of plants. Phytoestrogens have structural and biological functions similar to the female sex hormone 17-β-estradiol as they bind to the estrogen receptors and function as agonists or antagonists. Phytoestrogens are used usually for reducing symptoms of menopause such as hot flush and osteoporosis. Also, they are used in lowering the risk of cardiovascular diseases, obesity, metabolic disorders, diabetes, and various types of cancer. Phytoestrogen has also shown neuroprotective effect on various neurological diseases may be due to structural similarity with estrogen which plays an important role in brain health and aging process **[5, 6]**. Estrogen receptor activation in brain areas affects the levels of neurotransmitters and cognitive functions **[6]**.

One of these phytoestrogens is biochanin A(BIO-A),(5,7-dihydroxy-4'-methoxy

isoflavone), which is found in red clover, soy, and cabbage [7]. BIO-A exists as an aglycon and exerts various biological activities by binding DNA and some proteins or as a competitive substrate to enzymes **[8]**. BIO-A is metabolized to the isoflavone genistein which has also several important biological activities **[9, 10]**. BIO-A was found included in many commercial supplements that contain isoflavones **[11]**. These supplements are available as a tablet usually used to relieve menopause manifestations. However, BIO-A has poor water solubility and low bioavailability. Several studies act on improving that either by using liposomes or nanoparticles **[8]**.

Pharmacological and biological activities of BIO-A are well-documented as anticancer **[12]**, anti-inflammatory **[13]**, antioxidant **[14]**, anti-microbial **[15]**, hepatoprotective **[16]**, neuroprotective **[17]**, anti-diabetic **[18]**, anti-hypertensive **[19]** and anti-hyperlipidemic **[20]**. BIO-A also was reported to be useful in the prevention of bone loss in postmenopausal women **[21]**, and to have a gastroprotective effect **[22]**. BIO-A can be used as a pain killer as it inhibits fatty acid amide hydrolase **[23]**. BIO-A may act as a skin whitening agent **[24]**. BIO-A also acts as a selective Estrogen receptor modulator so it can be used as an alternative estrogen therapy **[25]**.

In our review, we will discuss the neuroprotective effect of BIO-A and the possible mechanisms in multiple neurological disorders.

2. The possible mechanisms of the neuroprotective effect of BIO-A

The upcoming section briefly discusses the potential mechanisms for the neuroprotective effect of BIO-A. A summary for these mechanisms is depicted in **Fig.1.**

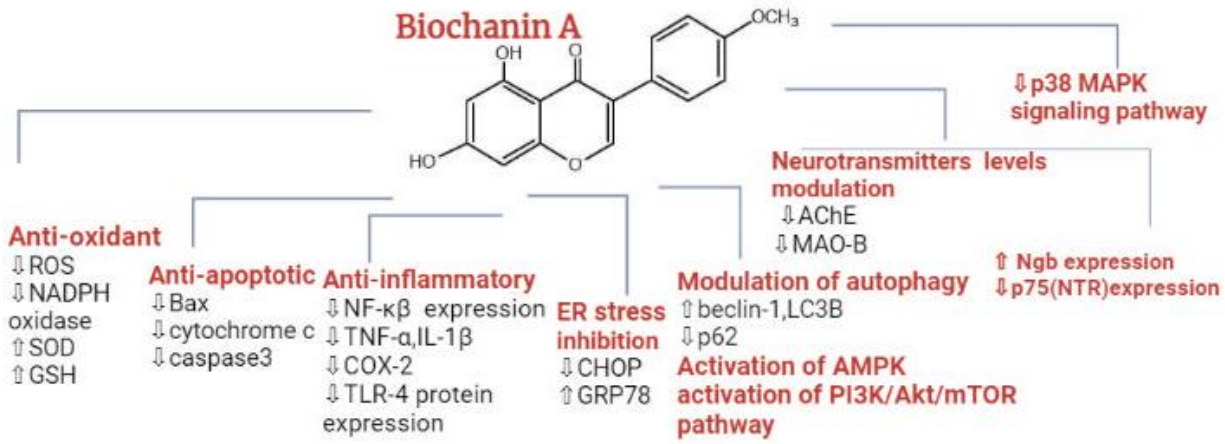

Fig.1: Molecular mechanisms of the neuroprotective effect of BIO-A

2.1. BIO-A as an antioxidant

Due to the erratic susceptibility of the brain to oxidative stress and knowing that excessive ROS causes neuropathology, oxidative stress is a therapeutic target for neurological disorders. It has been suggested that several neurological conditions and neurological disorders such as Alzheimer's and Parkinson's are triggered by oxidative stress-induced neuronal cell death **[26]**. Various types of ROS are the main mediators of oxidative stress **[27]**. The delicate equilibrium between ROS generation and clearance is interrupted during oxidative stress, which causes ROS to build up inside the cell **[27, 28]**. Elevated ROS levels within the cell cause neuronal damage by boosting lipid peroxidation, mitochondrial dysfunction, and apoptotic death of cells **[29]**. BIO-A has a protective effect on neurons as it lowers prooxidant levels and increases antioxidant levels in normal cells **[14, 30]**.

BIO-A also acts on intracellular oxidative stress as it boosts the expression of antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) **[31]**. These antioxidants initiate the conversion of the highly reactive superoxide to the less reactive hydrogen peroxide (H_2O_2) and also prevent lipid peroxidation **[32, 33]**. The cellular redox equilibrium is maintained by the fast interconversion of glutathione (GSH)to the glutathione disulfide (GSSG) **[34]**. BIO-A has been proven to prevent oxidative stress by boosting GSH **[35]**. BIO-A scavenges H_2O_2 in human cortical cell line HCN 1-A and restores neuronal viability in rat primary hippocampal neurons **[35, 36]**. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is considered necessary for the induction of the effects of antioxidants. As soon as it is activated, Nrf2 separates from Keap1 and proceeds to the nucleus, in which it unites with the anti-oxidant response element (ARE) and mediates the reduction process of heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO-1). Since SOD, GSH, and GST are produced in response to Nrf2 downstream signaling, oxidative stress-related cellular damage is prevented **[37]**. According to previous reports, BIO-A activates the Nrf2/ARE signaling pathway in human neuroblastoma cell lines (SH-SY5Y cells) to

protect them against the neurotoxicity caused by isoflurane **[38]**. Because BIO-A can improve the intracellular antioxidant response system by modifying the Nrf2 signaling pathway, it is also useful against the toxicity caused by arsenic **[37]**. BIO-A inactivates NADPH oxidase and MDA, as well as restoring superoxide dismutase, and glutathione peroxidase levels in a model of LPS-injected rats, a model of PD **[39]**.

2.2. BIO-A as an anti-apoptotic

Apoptosis is an innate form of cell death that is induced by both extrinsic and intrinsic pathways. The extrinsic pathway begins with Fas or TNF-α binding to FasR or TNFR death receptors. This causes death messages to be sent within the cell, stimulating caspase-8, which somehow induces caspase-3. Caspase-3 then starts a series of steps that triggers apoptosis. Whenever the cell is confronted with a wide range of external stimulation, such as radiation, toxins, hypoxia, and oxidative stress, the intrinsic pathway begins. It boosts mitochondrial membrane permeation and the release of protein molecules into the cell cytoplasm that enhances apoptosis. Such occurrences then invoke caspase-9, which stimulates caspase-3 and thus stimulates the execution pathway **[40]**. BIO-A was proven to protect neuronal cells by hindering both the extrinsic and intrinsic pathways and decreasing the levels of TNF-α, caspase-3, and caspase 8 **[17, 41]**. The BCL2 protein family has a significant impact on the regulation of the intrinsic apoptosis pathway. Pro-apoptotic proteins such as Bcl-10 and Bax, are up-regulated during apoptosis, whereas anti-apoptotic proteins like Bcl-2 and Bcl-x are down-regulated **[40]**. BIO-A treatment is linked to enhanced expression of anti-apoptotic proteins and down-regulation of pro-apoptotic proteins in normal cells **[17, 35]**.

2.3. BIO A as an anti-inflammatory agent

Activation of microglia cells is the first step in neuroinflammation **[42]**. JNK and NF-kB are primarily responsible for the activation of microglia. Pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) molecules aggravate the NF-kB signaling pathway via toll-like receptors (TLR). Cyclooxygenase-2 (COX-2), iNOS, and other pro-inflammatory cytokines are among the NF-κB downstream effector pathways. BBB disruption and neurodegeneration have been linked to elevated expression of pro-inflammatory cytokines (IL-1β, TNF- α, and prostaglandins). DAMP and PAMP detection by pattern recognition receptors (PRRs), like NLRP3, triggers the development of misfolded proteins and eventually contribute to the development of inflammasomes. Inflammasomes then results in the production of pro-inflammatory cytokines, like IL-1β, IL-18, and pyroptosis, which then result in severe inflammation **[3]**. The effects of BIO-A are summarized in **Table 1**.

BIO-A has been also shown to attenuate JNK and NF-κB so inhibiting neuroinflammation **[43, 44]**. BIO-A reduces the levels of pro-inflammatory cytokines in the cerebral ischemia-reperfusion model **[48]**. BIO-A also inhibits the NLRP3 inflammasomes **[45]**. Studies showed that BIO-A protected dopaminergic neurons against LPS by markedly decreasing microglial activation and reducing levels of NO, TNF-α, and superoxide **[47, 49]**. Another study revealed that BIO-A effectively diminished the LPS-induced generation of NO, NF-κB, TNF-α, and inflammatory cytokines **[50]**. A previous study on $AlCl_3$-induced neurodegeneration showed that administration of CPE,(chickpea extract, in which BIO-A is a major component), decreased the levels of inflammatory cytokines, oxidative damage, repressed amyloidosis, and decreased the expression of AChE **[51]**.

Table 1. BIO-A protects against neuroinflammation

Molecular target affected	BIO-A action	MODEL	Reference
NF-kB	Decreases levels of NF-KB	LPS Model	**[43]**
		SAH model	**[44]**
NLRP3	Inhibits NLRP3 inflammasome	Angiotensin II-induced damage of dopaminergic neurons	**[45]**
TNF-α	Decreases TNF-α levels	LPS model	**[46, 47]**
NO	Decreases NO expression		
Superoxide	Decreases superoxide levels		

2.4. BIO–A as ER stress modulator

Endoplasmic reticulum (ER) stress is a result of ER structural and functional abnormalities, including the buildup of misfolded proteins and changes to calcium homeostasis. Changes in certain proteins that result in translational attenuation, the stimulation of ER chaperones, and the destruction of misfolded proteins are characteristics of the ER response. Cellular signals that cause cell death are activated in cases of persistent or intensified ER stress. ER stress has been implicated in a variety of human neurological diseases such as PD, AD, and prion diseases **[53]**. BIO-A has been shown in rat models to reduce cerebral ischemic/reperfusion injury in rat models by repressing ER stress, cell death, and the p38 MAPK signaling pathway. And also BIO-A intervention was correlated with lower CHOP levels and higher GRP78 levels **[54]**.

2.5. BIO-A modulates Autophagy, PI3K/Akt/mTOR, and AMPK signaling

In response to nutritional and bioenergetic stressors, macroautophagy is a significant, evolutionarily conserved mechanism that can eliminate aggregated proteins and damaged organelles like mitochondria. This has sparked a lot of interest in treatments for neurological illnesses like Huntington's, AD, PD, and stroke which involve autophagy **[55]**. The regulation of signal transmission and biological processes such as cell proliferation, death, metabolism, and

angiogenesis is greatly influenced by the PI3 K/AKT/mTOR signaling pathway. Numerous human disorders, such as ischemic brain injury, neurodegenerative diseases, and tumors involves alterations in the PI3K/AKT/mTOR signaling pathway **[56]**. Modulation of the mTOR pathway protects against neuronal apoptosis and excessive autophagy. Both death programs were induced by oxidative stress and neuroinflammation in PD **[57]**. According to studies, BIO-A increases the autophagy marker beclin-1 and induces PI3K/Akt/mTOR signaling leading to protecting the nigral dopaminergic neurons in rotenone parkinsonian mice **[57]**.

A crucial metabolic regulator known as AMPK works to control several aspects of mitochondrial biology and homeostasis. The ovariectomized double transgenic (OVX-APP/PS1) mouse model's hippocampus had a relatively low level of AMPK activation. BIO-A treatment restored AMPK activation in that model. Such activation resulted in improvement of cognition, reducing Amyloid beta accumulation, and restoration of normal levels of mitochondrial biogenesis, dynamics, and mitophagy. BIO-A upregulates Beclin1, LC3B, Pink1, and Parkin, and reduces the expression of p62 **[58]**.

2.6 BIO-A act on Neurotransmitters levels modulation

In the synaptic cleft, the enzyme acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine into choline and acetate **[59]**. Acetylcholine esterase inhibitors are widely used as a therapeutic approach for AD **[60]**.

BIO-A decreases acetylcholinesterase activity of the whole brain in aged mice, and in mice treated with scopolamine. BIO-A also prevented the rise in noradrenaline and dopamine, which causes dementia **[61]**.

The enzyme monoamine oxidase (MAO) has an important effect on the metabolism of monoamine neurotransmitters and other amines. Hydrogen peroxide (H_2O_2), a mediator of oxidative stress, is produced by oxidative deamination that is catalyzed by MAO. Monoamine oxidase (MAO) exists in two forms, monoamine oxidase-A (MAO-A) and monoamine oxidase-B (MAO-B), each of which is encoded by a separate gene, and has a distinctive tissue allocation system and separate substrate specificity. Neurotransmitters like dopamine are deactivated by MAO-B **[62]**.

In contrast to healthy brains, the hippocampus and cerebral cortex of Alzheimer's disease brains have increased MAO-B expression **[63]**, and reactive astrocytes surrounding amyloid plaques contain an increased level (more than 3-fold) of active MAO-B **[64]**. As a result, inhibiting MAO-B expression might reduce oxidative damage and neuronal death, thereby slowing the disease's course **[65]**.

Monoamine oxidase-B (MAO-B) inhibitors are also frequently utilized in the treatment of PD **[62]**. BIO-A was found to be a powerful, reversible, and selective MAO-B inhibitor, and it might be strongly suggested for further research into its potential use in the treatment and prophylaxis of PD and AD **[66]**.

2.7. BIO-A as a MAPK signaling inhibitor

Mitogen-activated protein kinase (MAPK) is involved in the response to various stimuli. Additionally, members of the MAPK family are crucial in regulating how cells react to cytokines and stressors **[67]**. MAPK phosphorylation has been linked to the regulation of proinflammatory cytokines in LPS-stimulated microglia. ERK1/2, JNK, and p38 were phosphorylated as a result of the activation of MAP kinases by inflammatory cytokines and oxidative stress. MAPK inactivation is a possible mechanism of the anti-

inflammatory implication of BIO- A in parkinsonian rats, and also in BV2 microglial cells **[43, 47]**.

Iron and methyl phenyl tetrahydropyridine (MPTP) co-treatment resulted in neurochemical and behavioral decline by triggering microglial p38 MAPK. BIO-A alleviates behavioral and neurochemical decline by inhibiting microglial p38 MAPK **[68]**. BIO-A also protects neuronal cells in cerebral ischemia/reperfusion by inhibiting neuroinflammation and inhibiting MAPK p38 **[48]**.

2.8. BIO-A increases neuronal cell viability

Up-regulation of the p75 (NTR) death receptor starts the signaling pathways that lead to cell death following spinal cord injury or distal polyneuropathy. *In vitro* studies on BIO-A showed effective attenuation of p75 (NTR) expression induced by ibuprofen and enhancement of the survival of CCFSTTG1 and U87MG cancer cells pre and post-p75 (NTR) induction. Besides, this study proved that the inhibitory effect of BIO-A on p38-MAPK contributes to in cell viability **[69]**.

Neuroglobin (Ngb) is a globin that shares resembles hemoglobin and myoglobin in structure. According to *in vivo* studies increased Ngb levels considerably protect the brain and heart from hypoxia, ischemia, and oxidative stress bad effects, while decreasing Ngb expression exacerbates tissue damage. Human Ngb up-regulation may guard nerve cells against mitochondrial dysfunction and neurological disorders like AD **[70]**. BIO-A has been shown to up-regulate Ngb in both mice and humans **[71]**.

3- BIO-A role in neurological disorders

The role of BIO-A in different neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), stroke, multiple sclerosis (MS), brain injury, glioblastoma, and depression is depicted in **Fig. 2.**

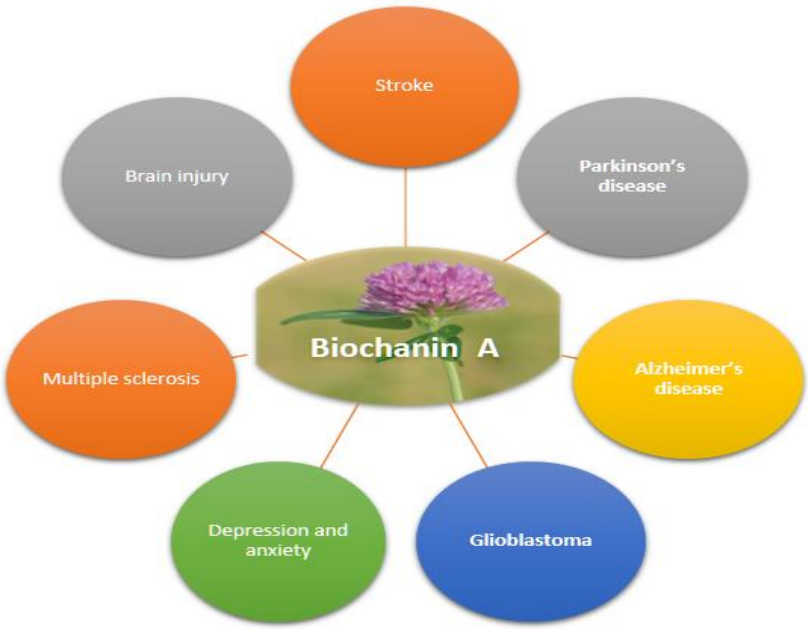

Fig. 2: BIO-A has a neuroprotective effect in multiple neurological disorders

3.1. BIO-A investigation in AD

Among the most prevalent progressive neurodegenerative disorders is AD which is distinguished by dementia. Amyloid-beta (A-beta) oligomerization and tau protein hyperphosphorylation are thought to be key pathogenic features of AD. These aberrant protein aggregates cause several physiological reactions, including neuroinflammation, mitochondrial dysfunction, epigenetic modifications, and blood-brain barrier abnormalities, and ultimately result in neuron degeneration **[72]**.

BIO-A has been demonstrated to have positive effects on Alzheimer's disease. BIO-A can be used as a protective and as a possible treatment for Alzheimer's disease. It binds the constructed fibril configuration of amyloid$_{25-35}$ and prevents apoptosis caused by -amyloid$_{25-35}$ through inactivation of the caspase enzyme **[17, 73]**. Additionally, it was shown that BIO-A inhibits BACE 1, which has been linked to the abnormal creation of the Aβ peptide, a key player in the etiology of Alzheimer's disease. **[74]**.

3.2. BIO-A investigation in PD

Patients with PD suffer from symptoms like bradykinesia, stiffness, tremors, cognitive impairment, and depression. PD is characterized by dopaminergic neuron degeneration in the substantia nigra **[75]**.

Experiments show that BIO-A has a positive impact on PD by inhibiting the inflammatory response and the MAPK signaling pathway, decreasing levels of inflammatory cytokines and TNF-α. It also inhibits the phosphorylation of ERK, JNK, and p38 in the substantia nigra of parkinsonian rats **[47]**. BIO-A considerably reduced MDA and enhanced GSH in the substantia nigra of rats treated with iron and rotenone. Iron and rotenone co-treatment may worsen neurochemical and cognitive decline by triggering redox imbalance, and elevated iron supplements in newborns may contribute to the etiology and pathogenesis of Parkinson's disease. Furthermore, BIO-A protects dopaminergic neurons by establishing redox equilibrium **[31]**. BIO- A acts as an anti-apoptotic against L-glutamate-induced cytotoxicity in a PC12 cell line **[41]**.

3.3. BIO-A investigation in stroke

In both developed and developing nations, both ischemic stroke and hemorrhagic stroke, continues to be the leading cause of mortality and disability **[76]**. Although acute stroke treatment aims to restore blood flow, this can worsen ischemia/reperfusion (I/R) injury-related neuronal degeneration **[77]**. The pathophysiological damaging processes of cerebral I/R include energy failure, excitatory neurotransmitter release, disruption of the BBB, Ca^{2+} accumulation inside cells, oxidative stress, inflammation, and apoptosis **[78]**. As a result, developing neuroprotective agents, maybe a successful plan of action for treating ischemic stroke **[79]**. BIO-A has been demonstrated to reduce the inflammatory injury caused by cerebral ischemia/reperfusion by inactivating the P38MAPK signaling pathway **[48]**. Another study found that BIO-A greatly reduced infarct size, reduced brain edema, and considerably improved neurological deficits. It also boosted SOD and GSH while decreasing MDA formation. Furthermore, in ischemic brain injury, BIO-A enhanced nuclear translocation of Nrf2, and increased HO-1 production, while inactivating NF-KB **[80]**.

Excitotoxic glutamate concentrations contribute to neuronal cell death in the context of cerebral ischemia. Glutamate oxaloacetate transaminase (GOT) degrades deleterious glutamate in the brain of stroke patients. BIO-A mitigates the injury by enhancing the expression of GOT **[81]**.

3.4. BIO–A investigation into multiple sclerosis

Multiple sclerosis (MS), a chronic inflammatory and neurodegenerative disorder of the central nervous system that results in damage of the myelin sheath, axonal degeneration, and neuronal death. Up to 70% of patients suffer from cognitive decline in form of loss of memory, which is a prominent feature of the disease. As a result, it significantly affects patients' quality of life. In contrast to cuprizone (CPZ), BIO-A significantly improved mice's spatial and recognition memories in behavioral tests. BIO-A alleviated neuronal injury in the hippocampus. **[82]**.

3.5. BIO-A investigation in brain injury

Early brain injury after subarachnoid hemorrhage (SAH) is a consequence of inflammatory injury and neuronal apoptosis. Recently, it has been demonstrated that reducing inflammation can lessen post-SAH neuronal death and behavioral impairment. BIO-A hamper the TLRs/TIRAP/MyD88/NF-kB pathway to lessen inflammatory damage and neuronal death following subarachnoid hemorrhage **[44]**.

Traumatic brain injury (TBI) is the leading cause of serious morbidity and disability in individuals under 45 years old. Many TBI survivors do not fully recover and still show evidence of persistent disability, despite breakthroughs in research and clinical studies of TBI remedies. Diffuse axonal damage (DAI) and cerebral white matter degeneration are characteristics of diffuse traumatic brain injury (TBI), which affects the brain and brainstem. Previous research has shown that BIO-A suppresses apoptosis and gliosis, attenuates BBB damage, and swelling, and improves behavioral functions in TBI in its initial stages **[83]**.

3.6. BIO-A investigation in Glioblastoma

Glioblastoma is a prevalent malignant primary brain cancer, with an average survival period of 14.6 months and a 5-year survival rate less than 5.5 percent **[84]**. Patients with glioblastoma are routinely treated with radiation, temozolomide (TMZ) chemotherapeutic agent, and the safest possible neurosurgical excision. The fact that present treatment methods frequently have negative consequences has made the development of new non-toxic therapeutic approaches a top research priority **[85]**.

In vitro and in vivo studies have demonstrated that BIO-A has potent anti-tumor properties by increasing ROS inside the tumor cells while reducing aerobic glycolysis in glioma cancer cells. Additionally, BIO-A acts by regulating hypoxia-inducible factor 1(HIF-1a) production via inactivating the AKT/mTOR signaling pathway. BIO-A treatment significantly reduces the Warburg effect in the cell line of human glioma, U251 **[86]**.

3.7. BIO-A investigation in depression and anxiety

Depression is a prevalent condition that is linked to a lower quality of life, as well as an increase in morbidity and death **[87]**. For women, depression is a real concern; in fact, it was recently featured as one of the top 11 health problems affecting pre-and postmenopausal women **[88]**.

Isoflavones found in soybeans in physiologically significant levels. Clinical studies attest to soybean's potent antidepressant properties **[89]**. MF11RCE, in which formononetin, genistein, biochanin A, and daidzein isoflavones are presented in a specific ratio, was proven clinically to be successful in alleviating depression and anxiety symptoms in postmenopausal women **[90]**.

Conclusion

As a powerful phytoestrogen, BIO-A has attracted a lot of research interest. Through its anti-oxidant, anti-inflammatory, anti-apoptotic, regulation of autophagy pathway, and ER stress modulation properties, BIO-A has been shown to have broadspectrum neuroprotective effects. Also, BIO-A acts as MAO inhibitor and AchE modulator as well as an inactivator of the P38 MAPK pathway, and up regulator of Ngb. The neuroprotective impact of BIO-A was reported in a variety of neurological disease models, including AD, PD, and glioblastoma. However, clinical trials are essential to verify the potential neuroprotective utility of BIO-A and extend the benefit to patients.

Declarations

Ethics approval and consent of participation

Not applicable

Consent of publication

Not applicable

Data and materials availability

All data produced or analyzed throughout this study are included in the current manuscript.

Competing interests

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Authors' contributions

Sarah A.Hussein: Writing - original draft, manuscript revision. Mai F.Tolba: review idea and outline & editing, Supervision. Haidy E.Michel: review & editing, Supervision. Samar S.Azab: review & editing, Supervision, all authors approved the final manuscript.

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