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Protective Effect of Natural Products against Huntington's Disease: An Overview of Scientific Evidence and Understanding Their Mechanism of Action

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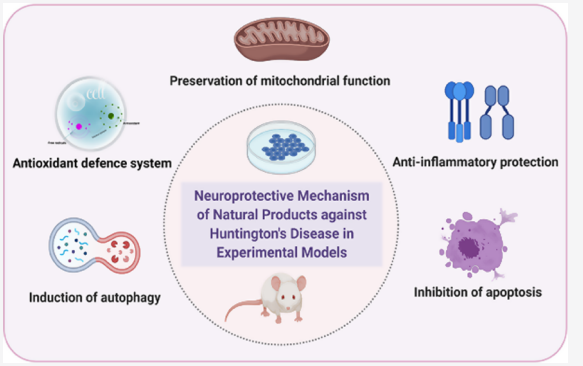
ABSTRACT: Huntington's disease (HD), a neurodegenerative disease, normally starts in the prime of adult life, followed by a gradual occurrence of characteristic psychiatric disturbances and cognitive and motor dysfunction. To the best of our knowledge, there is no treatment available to completely mitigate the progression of HD. Among various therapeutic approaches, exhaustive literature reports have confirmed the medicinal benefits of natural products in HD experimental models. Building on this information, this review presents a brief overview of the neuroprotective mechanism(s) of natural products against *in vitro*/*in vivo* models of HD. Relevant studies were identified from several scientific databases, including PubMed, ScienceDirect, Scopus, and Google Scholar. After screening through literature from 2005 to the present, a total of 14 medicinal plant species and 30 naturally isolated compounds investigated against HD based on either *in vitro* or *in vivo* models were included in the present review. Behavioral outcomes in the HD *in vivo* model showed that natural compounds significantly attenuated 3-nitropropionic acid (3-NP) induced memory loss and motor incoordination. The biochemical alteration has been markedly alleviated with reduced lipid peroxidation, increased endogenous enzymatic antioxidants, reduced acetylcholinesterase activity, and increased mitochondrial energy production. Interestingly, following treatment with certain natural products, 3-NP-induced damage in the striatum was ameliorated, as seen histologically. Overall, natural products afforded varying degrees of neuroprotection in preclinical studies of HD via antioxidant and anti-inflammatory properties, preservation of mitochondrial function, inhibition of apoptosis, and induction of autophagy.



KEYWORDS: *Huntington's disease, natural products, neuroprotective, neurodegenerative, 3-nitropropionic acid, herbal medicine*

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant, inherited neurological disorder associated with a pathogenic expansion of cytosine-adenine-guanine (CAG) trinucleotide repeats in exon 1 of the huntingtin gene (*Htt*).[1] The onset of HD occurs between 30 and 50 years (mean survival, 15−20 years). HD occurring before 21 years of age is deemed as a juvenile HD, while the other extreme, "late-onset", is that occurring after 60 years old.[2]

The data from meta-analyses have reported a global prevalence of approximately 2.7 in 100 000 HD with the lowest incidence seen among Asians and the highest in Western populations.[3] However, the overall incidence of HD worldwide remains unclear because epidemiological evidence from Asia and African populations to date are limited to only clinical reviews and case studies.[4] Despite discerning common HD genetic origins, disease progression tends to vary based on an individual's mutation rates, diagnostic stigma, and criteria.

Mutant Htt (mHtt), a mutated functional protein carrying abnormal and elongated polyglutamine (polyQ) is the main factor in contributing to the development of HD, exhibiting putative detrimental properties to the entire parts of the brain.[2] Pathologically, a toxic gain function of mHtt tends to be devasting to the intracellular pathways in altering proteostasis and protein degradation following transcription and synaptic dysfunction, striatal excitotoxicity, dopamine toxicity, mitochondrial dysfunction, metabolic impairment, oxidative stress, and neuronal cell death.[5] After a premanifest period of HD, its clinical characteristics are diagnosed based on behavioral components, including cognitive and motor signs and symptoms. Paradoxically, there are merely symptomatic treatments often used in ameliorating the negative impacts of

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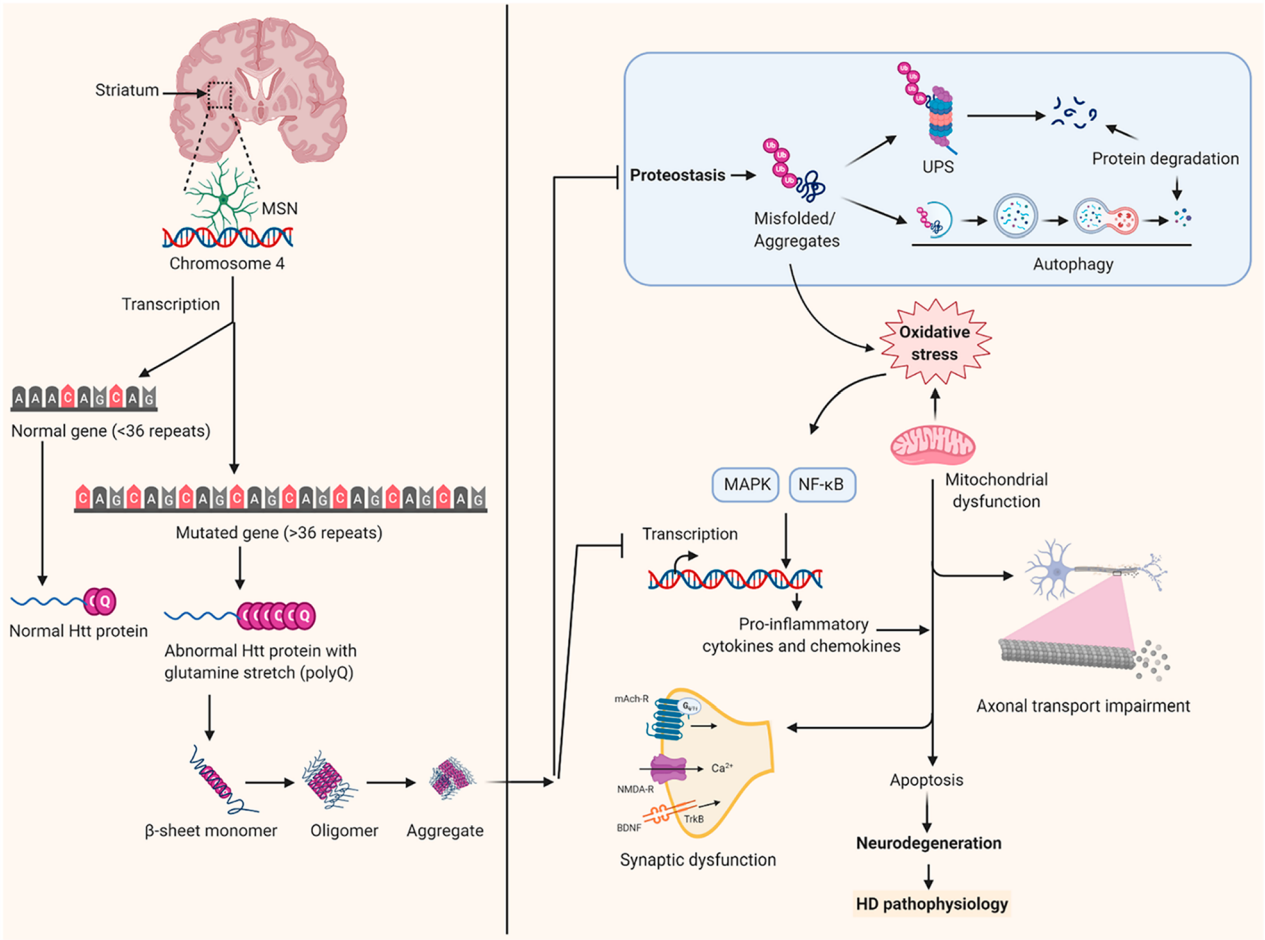

Figure 1. Pathophysiology of HD. Mutation of *Htt* characterized with repeat expansion of CAG trinucleotides is the key factor in HD. Abnormal aggregation of mutant Htt protein may cause toxic effects in neurons, leading to a series of pathogenic mechanisms associated the alteration in proteostasis and protein degradation following mitochondrial dysfunction, oxidative stress, transcription and synaptic dysfunction, axonal transport impairment, and a series of metabolic impairments subsequent to neurodegeneration. Abbreviations: HD, Huntington's disease; *Htt*, huntingtin gene; MAPK, mitogen-activated protein kinase; MSN, medium spiny neurons; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; UPS, ubiquitin proteasome system.

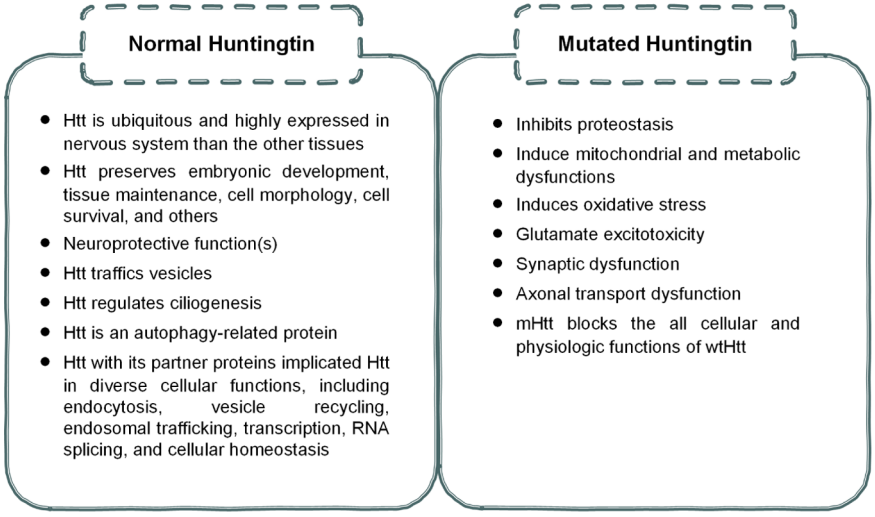

Figure 2. Normal versus mutated huntingtin. Abbreviations: Htt, huntingtin; mHtt, mutant huntingtin; wtHtt, wild-type huntingtin.

HD. Therefore, researchers are oriented toward implementing therapeutic strategies by targeting the pathway of mHtt production to address the cause of HD pathophysiology. Nevertheless, in defiance to the existing therapeutic approaches, HD impact is still not fully controlled.

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To overcome the above-mentioned concerns, investigation has been devoted to the isolation of novel compounds from a variety of natural products in modulating relevant neurodegenerative disorders. Up until then, a plethora of traditional treatments based on natural products have been shown to possess a wide range of therapeutic benefits for HD under *in vitro* and *in vivo* models. Indeed, based on relevant studies, natural products offer neuroprotection in experimental models predominantly through the antioxidant defense system, scavenging free radicals, neutralization of reactive oxygen species (ROS), reduction of oxidative stress, preservation of mitochondrial function, anti-inflammatory protection, inhibition of apoptosis, and induction of autophagy. To gain insight into the potential role of natural products, an overview of various natural products against HD in preclinical studies is presented in this review. We attempt to outline the pathophysiology of HD, mapping the clinical manifestations and current therapeutic approaches for HD. Subsequently, a summary of information gathering the neuroprotective potency of natural products against HD animal models is presented. Possible neuroprotective mechanisms are also highlighted and discussed based on the relevant findings from HD *in vitro* and *in vivo* models.

HUNTINGTON'S DISEASE

Pathophysiology of HD. The pathophysiology of HD is presented in Figure 1. Huntingtin (Htt) is a protein formed by more than 3100 amino acids, encoded by a gene located at chromosome 4.[2] Underlying the molecular mechanism, mutation of *Htt* gene characterized by the repeat expansion of CAG trinucleotides within exon 1 is the major factor in the pathophysiology of HD[6] (Figure 2). The number of the CAG repeats is predominantly chosen as the primary determinant of HD severity due to its instability and change in length with either a decrease of 1−2 units or an increase of 1−4 units during parental transmission.[2,6] For the normal population, the repeat is said to be polymorphic in the range of 6−35 units. Clinically, an individual with CAG repeats of 36−39 units has HD with incomplete penetrance correlated with later onset. They live a normal lifespan with no symptoms apparent for diagnosis. However, when the CAG repeats expand to above 40 units, it is considered as the longest repeats with high penetrance triggering signs of early onset diagnostic for HD. The stretched tract of polyQ at the amino terminus of the translated Htt protein is linked with the abnormal aggregation of protein and a complex loss of function phenotype.

The expanded polyQ tract with a high order of amyloid fibers and insoluble β-pleated sheets is prone to aggregation that can disrupt the intracellular function directly by affecting proteostasis and impairing protein degradation, thus resulting in transcription dysregulation.[2,7] The formation of protein aggregates in HD is a complex process encompassing sequestering of other proteins into the mHtt aggregates. Based on the said hypothesis, misfolded Htt cannot bind and inhibit the activation of caspase-3. N-terminal fragments produced from the caspase-3-mediated proteolysis of mHtt tend to induce proteolytic cleavage and additional protease aggregation. Interestingly, proteasome components and heat shock proteins (HSPs) may form aggregates of mHtt.[7] Subsequently, the functional proteasomes and heat shock protein 70 (Hsp70) in neurons are progressively depleted *in vitro*, leading to a proteostasis collapse, accompanied by the chronic expression of mHtt and increased protein aggregation.[8] On the other hand, imbalance of the ubiquitin−proteasome system (UPS), which occurs due to the overwhelming proteostasis induced by mHtt, was observed in the brain tissue of mouse models and HD patients, thus suggesting that impaired protein degradation pathways may be involved.[5]

Transcriptional dysregulation has been reported to be linked with progressive expression of mHtt in HD. Studies[2,7] have shown that various transcriptional factors, including specific protein-1 (Sp1), cyclic-adenosine monophosphate (cAMP) response element-binding protein (CREB)-binding protein (CBP), thymidine-adenine-thymidine-adenine (TATA)-binding protein (TBP), p53, and brain-derived neurotrophic factor (BDNF), tend to interact with *mHtt* while expressing mHtt. On the other hand, the level of CBP is decreased parallel to histone hypoacetylation as well as inhibition of CBP-regulated transcription. In addition, *Sp1* gene transcription is also inhibited as confirmed by the reduction of Sp1 binding onto specific promoters purported to be due to dissociation of binding of Sp1 from the particular promoter on *mHtt*. Moreover, suppression of the *BDNF* gene contributed by mHtt leads to striatal neuron degeneration.

Additionally, it is suggested that neutrophil aggregation caused by mHtt affects axonal transport by physically blocking the signal transport within the axon terminus leading to impairment of neuronal synaptic transmissions.[9] The expression of pre- and postsynaptic neurotransmitter receptors is also perturbed, contributing to synaptic dysfunction.[10,11] Besides intracellular dysfunction, various neurodegenerative mechanisms, including cortico-striatal excitotoxicity,[12] mitochondrial dysfunction,[13] metabolic energy impairment, and oxidative stress,[14] followed by cell death as a result of apoptosis and dysregulated autophagy, have been linked to HD pathophysiology.[2]

Interestingly, wild-type Htt (wtHtt) contributes to normal embryonic development. However, polyglutamine expansion obstructs the interaction of wtHtt with postsynaptic density 95 (PSD-95), leading to (1) the sensitization of *N*-methyl-D-aspartic acid (NDMA) and kynurenic acid (KA) receptors[12] and (2) glutamate-mediated excitotoxicity. mHtt also induces the tyrosine phosphorylation of NDMA receptors, promoting further sensitization.[15] In addition, as confirmed by Tang et al.,[16] the expression of metabotropic glutamate receptor subtype 5 (mGluR5) and NMDA receptor subtype 2B (NR2B) is involved in the mHtt-induced excitotoxicity in striatal medium spiny neurons (MSNs) of yeast artificial chromosome (YAC) transgenic mice.

Collectively, chronic NDMA activation and striatal dopamine (DA) depletion synergistically induce an increase in intracellular calcium levels, causing mitochondrial dysfunction.[16] Several studies have supported the fact that mitochondrial dysfunction and metabolic impairment are important pathological hallmarks of HD.[2,6] *In vitro* studies indicated the loss of mitochondrial function caused by 3-nitropropionic acid (3-NP) via the expression of cytochrome *c* concomitant with caspase activation eventually.[17,18] The release of cytochrome *c* triggers caspase activation, causing cleavage of mHtt and its translocation into the nucleus. According to Bae et al.,[19] nuclear mHtt can induce the expression of p53 (a vital transcription factor, which mediates the expression of several mitochondrial proteins, including Bax) in the primary neuronal cultures from mouse embryos, leading to mitochondrial abnormalities. Additionally, it has been suggested that mHtt fragments can associate with the

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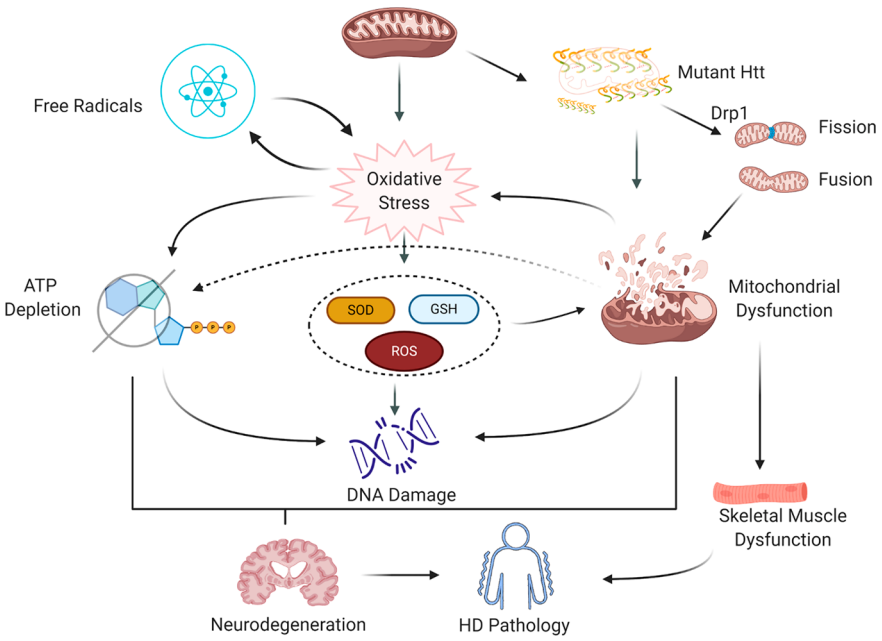

Figure 3. Interaction of mutant Htt with mitochondrial protein and subsequent pathogenic changes in HD neurons. Abbreviations: ATP, adenosine triphosphate; Drp1, dynamin-1-like protein; GSH, reduced glutathione; HD, Huntington's disease; Htt, huntingtin; ROS, reactive oxygen species; SOD, superoxide dismutase.

outer mitochondrial membrane by causing the opening of mitochondrial permeability transition pores and disrupting the electron transport chain (ETC).[19]

Two important but opposing forces that maintain mitochondrial shape and structure are mitochondrial fission and mitochondrial fusion. The plethora of evidence suggests that mitochondrial dynamics become disturbed in neurodegenerative diseases like HD. In the aged neurons that express mutant proteins, such as mutant Htt, an imbalance between fission and fusion leads to abnormalities in mitochondrial structure and function and neuronal damage. Figure 3 shows mutant huntingtin interaction with the mitochondrial protein dynamin-1-like protein (Drp1) and subsequent pathogenic changes in HD neurons. Overall, available evidence suggests that Drp1 intermingles with mutant Htt and increases Drp1 enzymatic activity in HD-affected regions, leading to both synaptic and neuronal damage. The mitochondrial dysfunction is the key that leads to oxidative stress and hence low synaptic ATP. In connection to this, the increased oxidative stress activates inducible nitric oxide synthase (iNOS) expression and inhibits the endogenous enzymatic antioxidants, including superoxide dismutase (SOD), glutathione (GSH) peroxidase, and cAMP, all of which lead to protein and lipid peroxidation as well as DNA mutation. Subsequently, this leads to neuronal damage and loss, which are key hallmarks of HD.

Clinical Manifestation of HD. The course of the life of a person with one parent with HD can be divided into at-risk, preclinical, and clinical stages.[20] The presence of elongated CAG repeats indicates that the individual is in the "at-risk" stage. On the other hand, the presence of a *mHtt* gene, predisposes an individual to progress to the preclinical followed by the clinical stages. The clinical features of HD predominantly comprise behavioral components, including cognitive and motor signs and symptoms. In the early stage, psychiatric disturbance is the most frequent symptom, appearing before the onset of the motor symptoms. Nevertheless, behavioral symptoms vary between different patients although the signs and symptoms commonly negatively impact daily functioning and relationships.

Among the various psychiatric symptoms, irritability is deemed as the first. Retrospectively, the symptoms may also occur in any of the stages of HD. Irritability is expressed in different forms, including showing severe conflict with others or worse, physical aggression. Both irritability and aggression are usually caused by compulsion and obsession with an individual's lifestyle. Commonly, depression includes anxiety, and guilt and low self-esteem are present throughout the different HD stages. Apathy is difficult to distinguish from depression. Nevertheless, HD patients with apathy commonly express passive behavior in lieu of a lack of interest. As the disease progresses, psychosis may appear concomitant with a cognitive decline.[20]

Significant cognitive changes are one of the noticeable signs of HD and exist long before the inception of the first motor symptoms. Particularly, a patient's memory is impaired although language ability is comparatively spared. Subsequently, psychomotor processes tend to be more severely retarded. Additionally, cognitive decline is also associated with a series of unique functions, including an inability to plan or organize life single-handedly due to loss of mental agility. Owing to this fact, patients are incapable of making good mental judgments.

Motor incoordination is involuntary. Initially, the unwanted movements tend to affect the distal extremities, including the fingers and the toes as well as small facial muscles. Gradually, the defect spreads to the muscles, starting from the distal area to more and more proximal and even toward axial, thus making daily walking gait unstable and a challenge. In fact, dystonia, characterized by a slower movement accompanied by an increased tone of muscles and culminating in abnormal postures, is the first motor symptom in HD.[21]

Among all the stated motor symptoms, choreatic movements, particularly continuous movements of the eyes,

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Figure 4. Medicinal plants with potential against HD in experimental models.

Table 1. List of Natural Products (Medicinal Plants) with Potential against HD in Experimental Models

natural plants					
botanical name	common name	family name	part of plants	natural compounds	refs
Anemarrhenae asphodeloides		Asparagaceae	rhizome	mangiferin	Piwowar et al.[33]
Calendula officinalis	marigold	Asteraceae	flower		Shivasharan et al.[45]
Celastrus paniculatus	jyotishmati, malkangni or kangani	Celastraceae	seed		Malik et al.[47]
Centella asiatica	Gotu Kola	Umbelliferae	leaf		Shinomol et al.[50]
Convolvulus pluricaulis	Shankhpushpi	Convolvulaceae	whole plant	scopoletin	Kaur et al.[54]
Ficus religiosa	pimpala or pipal tree	Moraceae	leaf		Bhangale et al.[58]
Ginseng radix	Korean red ginseng	Araliaceae	root	ginsenosides	Jang et al.[60]
Luehea divaricata	acoita-cavalo	Tiliaceae	leaf		Courtes et al.[63]
Panax quinquefolius	American ginseng	Araliaceae	leaf and stem	panaxadiols (Rb_1, Rb_3, Rd)	Lian et al.[65]
Phoenix dactylifera	date palm	Arecaceae	fruits		Essa et al.[36]
Psoralea corylifolia		Leguminosae	seed		Im et al.[39]
Punica granatum	pomegranate	Punicaceae			Al-Sabahi et al.[43]
Withania somnifera	Ashwagandha	Solanaceae	root		Kumar and Kumar[69]
Zingiber officinale	ginger	Zingiberaceae	root		Sharma et al.[71]

eyebrows, head, and tongue, exist consistently during the disease progression. Eventually, all HD patients will develop some forms of hypokinesia, slowly leading to bradykinesia (slowness of movement), followed by akinesia (difficulty in initiating movement). These altered motor performances progressively trigger an individual to have difficulties in standing and walking. Talking and swallowing problems are other more prominent motor symptoms for HD.[20] Having said that, the time when the signs and symptoms start to interfere with daily tasks are hugely dependent on the type of daily activities and work of HD patients.

Current Therapies in HD. To date, there are no promising treatments for the long-term unwanted effects of HD, which are being combated by symptomatic prevention and treatments for mitigating the psychiatric, cognitive, and motor deformities of HD. In the recent status of HD drug therapies, only tetrabenazine is approved by Food and Drug Administration (FDA) in the treatment of chorea and other HD-related motor symptoms.[22−24] Antipsychotic drugs such as tiapridal and olanzapine are believed to have the potential to treat chorea by modulating dopamine receptors. Their performances are currently being evaluated under a phase III clinical trial.[23,25]

Given the paucity of proven potency of existing symptomatic treatments for HD, the current targets of emerging treatments are focused on the development of mechanism-based therapies in parallel with the concepts and knowledge of possible pathways involved in HD pathogenesis. Therefore, growing therapeutic strategies have been aimed at Htt lowering, Htt modulation, immunomodulation, synaptic modulation, and stem cell transplantion approaches, which may be either RNA- or DNA-based.[3] In the former, antisense oligonucleotides (ASOs), synthetic single-stranded DNA sequences, and RNA interference (RNAi) can induce advanced degradation of mHtt to lower mHtt levels.[26] Normally, two types of ASOs are used

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Table 2. Summary of Natural Products (Medicinal Plants) with Potential against HD as Confirmed by *In Vitro* Studies[a]

natural plants (botanical name)	dose	IC_{50}	EC_{50}	cell lines	model	molecular outcomes	mode of action	refs
Anemarrhena asphodeloides	0.5, 1 μg/mL[b]	*c*	*c*	PC-12 cells	3-NP	cell viability ↑	antioxidant system	Piwowar et al.[33]
Phoenix dactylifera	1−1000 μg/mL	276.4 μg/mL	*c*	PC-12 cells	3-NP	cell viability ↑, ATP ↑, LDH activity ↓, ROS ↓, MDA ↓, NO ↓, SOD ↑, GPx ↑, GSH ↑	scavenge ROS	Essa et al.[36]
Psoralea corylifolia	10, 50, 100 μg/mL[b]	*c*	*c*	PC-12 cells	3-NP	cell viability ↑, ATP ↑, OCR ↑, mMP ↑; mitochondrial superoxide ↓	restoration of mitochondrial function	Im et al.[39]
Punica granatum	40 μM	*c*	*c*	PC-12 cells	genetically modified	ROS ↓, RNS ↓, MDA ↓, LDH activity ↓, NO ↓, LPO ↓	neutralize ROS, ↑ antioxidant gene expression	Al-Sabahi et al.[43]

[a]Abbreviations: 3-NP, 3-nitropropionic acid; $A_{2A}R$, adenosine A_{2A} receptor; AMPK, AMP-activated protein kinase; Atg, autophagy; ATP, adenosine triphosphate; CGNs, cerebellar granule neurons; LC3-I, cytosolic form of LC3; LC3-II, LC3-phosphatidylethanolamine conjugate; LDH, lactate dehydrogenase; LPO, lipid peroxidation; MDA, malondialdehyde; MEF, mouse embryonic fibroblasts; mHtt, mutant huntingtin; mMP, mitochondrial membrane potential; MSN, medium spiny neurons; mTOR, mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; OCR, oxygen consumption rate; PC-12, pheochromocytoma-12; PKA, protein kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species; WT, wild-type. [b]Concentration dependent. [c]Not determined.

(1) allele-specific ASOs, which target only *mHtt*, and (2) allele nonspecific ASOs targeting *wtHtt* and *mHtt*. Nevertheless, reduced levels of *wtHtt* and *mHtt* may confer potential risks of deleterious effects, making these therapeutic approaches not well-established. Currently, IONIS-HTT_{Rx}(RG6042), one of the allele nonspecific ASOs, is being tested for its safety in phase III clinical stages of development.[26]

On the other hand, the *mHtt* gene with elongated CAG repeats can be targeted by using (1) zinc finger motif proteins (ZFPs) to repress its transcription or (2) clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated system 9 (CRISPR-Cas9), which is a genome editing tool to edit mutated *Htt* DNA sequences.[27] To date, RNAi, ZFPs, and CRISPR-Cas9 are still in preclinical stages. Another useful alternative strategy is to modulate Htt homeostasis by inhibiting its aggregation or promoting its clearance. In fact, immunotherapy is likely to be a novel and promising approach to modulate the status of pro-inflammatory profile in HD patients. Despite negative findings from other disease-modifying clinical trials, immunotherapies via targeting semaphoring 4AD (SEMA4D) for modulation of neuroinflammation pathways still yield promising findings.[28] Finally, depending on an individual's disease progression, the development of surgical treatment such as stem cell transplantation has also been suggested.

RESULTS AND DISCUSSION

Protective Effects of Natural Products (Medicinal Plants) against HD. The neuroprotective effect of natural products in HD experimental models has been extensively studied. Hence, a variety of medicinal plants (Figure 4) that have been studied in preclinical models of HD is listed in Table 1, while a comprehensive detail of their neuroprotective effects against HD under *in vitro* and *in vivo* models are summarized in Tables 2 and 3, respectively.

In Vitro Studies. Anemarrhenae asphodeloides Bunge. The rhizomes of *Anemarrhenae asphodeloides* Bunge, which belongs to the Asparagaceae family, are a widely used traditional medicine in Eastern Asian countries such as China, Japan, Korea, and Mongolia. The rhizomes of *Anemarrhenae asphodeloides* are also well-known as Zhimu and Yanghuzi in Chinese medicine and Chimoi and Jimo in Japanese and Korean medicine, respectively.[29] According to Chinese medicine, *Anemarrhena* rhizome is recognized as a traditional warm herb for yin nourishing, heat-clearing, and kidney, lung, and stomach meridian entering correlated with the curative function of treating dry cough, fevers, night sweats, menopause syndrome, and diabetes.[29,30] It contains an abundance of active constituents, such as xanthones, saponins, alkaloids, flavonoids, anthraquinones, phenylpropanoids, and organic acids[31,32] with a series of pharmacological benefits, including anti-inflammatory, antibacterial, antipyretic, antiviral, antidiabetic, and anticoagulation.[29,33] A recent study was conducted by Piwowar et al.[33] to evaluate the potential protective effects of the *Anemarrhena* rhizome ethanolic extract toward a HD *in vitro* model with 3-NP induced neurotoxicity in pheochromocytoma (PC-12) cells. Suppression of 3-NP induced cytotoxic activity and enhancement of cell proliferation were observed in *Anemarrhena* rhizome extract-treated cells in which cell apoptosis and morphological changes were prevented. However, the protective action was dependent on the method of treatment, concentration, and incubation time. The finding reports that the xanthone fraction of ethanolic *Anemarrhena* rhizome extract (0.5 and 1.0 μg/mL) offered the protection for preincubated PC-12 cells in a concentration-dependent manner.

Phoenix dactylifera Linn. *Phoenix dactylifera* Linn (Arecaceae) is also known as date palm, a well-known woody fruit tree predominantly cultivated in Middle, Eastern, and Western Asia and North Africa. Date palm fruits are highly nutritious and rich in carotenoids, sterols, flavonoids, lignans, and phenolic acid, which are potent antioxidants.[34] They are edible fruits with high carbohydrates and lower tannins and moisture content. Pharmacological activities of date palm fruits including antiviral, anti-inflammatory, antifungal, anticancer, nephroprotective, hepatoprotective, and antihyperlipidemic properties have been extensively reported in previous studies.[35,36] Recently, it was reported to possess neuroprotective effects in a 3-NP induced HD *in vitro* model.[36] In the study, the ethanolic extract of date palm fruits was observed to ameliorate oxidative stress-induced mitochondrial dysfunction evidenced by restored intracellular ATP production in 3-NP intoxicated PC-12 cells. It was attributed to the antioxidant properties of the date palm fruits in reducing the ROS generation accompanied by an increase of endogenous enzymatic antioxidants (SOD and GPx) and nonenzymatic antioxidants such as reduced glutathione (GSH).[36] In addition, the results from cytotoxicity assay demonstrated that the

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Table 3. Summary of Natural Products (Medicinal Plants) with Potential against HD Confirmed by *In Vivo* Studies[a]

natural plants (botanical name)	dose (mg/kg); route	duration (days)	animal models, sex	toxin	outcomes: behavioral	outcomes: biochemical	outcomes: histopathological	mode of action	refs
Calendula officinalis	100, 200; po	7	Wistar rats, female	3-NP, ip	locomotor count ↑, TL ↓, body balance ↑, hind limb function ↑, grip strength ↑	LPO ↓; GSH ↑; TSH ↑; GST ↑; CAT activity ↑; NIT ↓	striatal degeneration ↓	antioxidant, anti-inflammatory, and estrogenic protection	Shivasharan et al.[45]
Celastrus paniculatus	100[b], 200; po	20	Wistar rats, male	3-NP, ip	impairment in grip ↓, locomotor activity ↑, TL ↓, TSTQ ↑	GSH ↑, SOD ↑, CAT activity ↑; MDA ↓, NIT ↓		↑ antioxidant defense system, ↓ glutamate toxicity	Malik et al.[47]
Centella asiatica	5, po	10	prepubertal mice, male	3-NP, ip		ROS ↓, MDA ↓; protein carbonyl ↓; GSH ↑, TSH ↑; SOD ↑, GST ↑, GPx ↑; MTT ↑, CS ↑; SDH activity ↑, MDH ↑; ETC enzymes acitivities ↑; Na^+, K^+ and ATPase ↑		antioxidant defense and preservation of mitochondrial integrity	Shinomol et al.[50]
Convolvulus pluricaulis	10, 20[b]; po	14	Wistar rats, male	3-NP, ip	locomotor, grip strength, and rotarod activity ↑	GSH ↑, SOD ↑; MDA ↓, NIT ↓		↑ antioxidant defense system	Kaur et al.[54]
	100, 200[b]; po	20	Wistar rats, male	3-NP, ip	locomotor and rotarod activity ↑, TL ↓	GSH ↑, SOD ↑, CAT activity ↑; MDA ↓, NIT ↓		↑ antioxidant defense system	Malik et al.[52]
Ficus religiosa	100, 200, 400[b]; po	14	Wistar rats, male	3-NP, ip	locomotor and muscle activity ↑	GSH ↑, SOD ↑, CAT activity ↑; AChE ↓; MDA ↓	swelling of cells ↓, density of cells ↑, neuronal damage ↓	↓ oxidative stress	Bhangale et al.[58]
Ginseng radix	50, 100, 250;[c] po	18	ICR mice, male	3-NP, ip		microglial activation ↓, expression of TNF-α, IL-1β, IL-6 and iNOS ↓, activation of JNK, ERK ↓, MAPKs and NF-κB ↓	lesion volume and striatal degeneration ↓	inhibition of MAPKs and NF-κB	Jang et al.[60]
Luehea divaricata	500, 1000; ig	10	Wistar rats, male	3-NP, ip	rotarod latency ↓	ROS ↓; TBARS ↓; AChE ↓; GSH/GSSG ratio restored		antioxidant defense system	Courtes et al.[63]
Panax quinquefolius	10, po	5	SD rats, male	3-NP, ip	behavioral change and motor impairment ↓		loss of neurons in the hippocampus ↓, striatal lesion ↓	scavenging free radicals	Lian et al.[65]
					hypothermia ↓, PPI deficit ↓, locomotor activity ↑	GABA ↑, DA ↑, 5-HT ↑, NE ↑; GSH ↑, MDA ↓	degree of pathological injuries in brain ↓	antioxidant defense system	Mahdy et al.[115]
Withania somnifera	100, 200;[c] po	14	Wistar rats, male	3-NP, ip	motor activity ↑, grip strength ↑	MDA ↓, NIT ↓; SOD ↑, CAT activity ↑; LDH activity ↓; NADH ↑, SDH activity ↑, MTT ↑		antioxidant defense system	Kumar and Kumar[69]
Zingiber officinale	100, 200;[c] po	7	Wistar rats	3-NP, ip	locomotor activity ↑, grip strength ↑, memory performance ↑	AchE ↓; MDA ↓; GSH ↑	brain inflammation ↓, necrosis ↓, gliosis ↓	anticholinesterase potency	Sharma et al.[71]

[a]Abbreviations: 3-NP, 3-nitropropionic acid; 5-HT, 5-hydroxytryptamine; $A_{2A}R$, adenosine A_{2A} receptor; AC, adenyl cyclase; AChE, acetycholinesterase; ATP, adenosine triphosphate; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CAT, catalase; CREB, cAMP response element binding protein; CS, citrate synthase; DA, dopamine; DARPP-32, dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32000; ELT, escape latency; ERK, extracellular signal-regulated kinase; ETC, electron transport chain; FST, forced swimming test; GABA, γ-aminobuytric acid; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, glutathione disulfide; GST, glutathione S-transferase; HO1, heme oxygenase-1; Hsp70, heat shock protein 70; Htt, huntingtin; ICR, Institute of Cancer Research; ig, intragastric gavage; ip, intraperitoneal injection; IL-1β, interleukin-1β; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; LPO, lipid peroxidation; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MDH, malate dehydrogenase; mHtt, mutant huntingtin; NAD, nicotinamide adenine dinucleotide; NADH, NAD + hydrogen (H); NE, norepinephrine; NIT, nitrite; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; po, per os, oral administration; PPI, prepulse inhibition; ROS, reactive oxygen species; sc, subcutaneous injection; SD, Sprague−Dawley; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TH, tyrosine hydroxylase; TL, transfer latency; TNF-α, tumor necrosis factor α; TSH, total thiols; TST, tail suspension test; TSTQ, time spent in target quadrant. [b]Most effective dose. [c]Dose dependent.

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morphological characteristics and viability of PC-12 cells were retained in cells treated with the date fruit extract. The finding suggests the ability of *Phoenix dactylifera* L. fruits (1−1000 μg/mL) to protect neuronal cells against 3-NP-induced oxidative stress and biochemical changes under the HD *in vitro* model.

Psoralea corylifolia Linn. *Psoralea corylifolia* Linn, which belongs to Leguminosae family, is a well-known herbaceous legume that grows in China, Korea, and India. From ancient times, it has been used to ameliorate various ailments in both Chinese and Ayurvedic medicines. There are approximately a hundred bioactive compounds isolated from *Psoralea corylifolia* with flavonoid, meroterpene, and coumarin groups identified as notable bioactive phytochemicals having useful anti-inflammatory, radio-modulatory, antitumor, antiparkinsonian, and dopaminergic neuroprotective properties.[37]

Previous *in vivo* studies have revealed that the seed extract of *Psoralea corylifolia*, furocoumarins, and psoralidin exert antidepressant effects.[38] In addition, it has been shown that *Psoralea corylifolia* seed extract (10, 50, and 100 μg/mL) attenuated 3-NP triggered mitochondrial dysfunction in PC-12 neuronal cells in a concentration-dependent manner.[39] It restored the mitochondrial function by increasing mitochondrial respiratory capacities accompanied by an increased basal oxygen consumption rate.

Punica granatum Linn. *Punica granatum* Linn (Punicaceae) or pomegranate is one of the oldest edible fruits originating from the Mediterranean and Middle East areas and also growing in the parts of North Africa and Asia.[40] Traditionally, *Punica granatum* has been used to treat kidney disorders, urinary infection, thyroid dysfunction, atherosclerosis, and cardiovascular diseases.[41] In Indian Unani medicine, *Punica granatum* is used to ameliorate diabetes mellitus. Due to its vast pharmacological activities, including anticancer, antioxidant, anti-inflammatory, antiulcer, and antilipoperoxidation,[40,42] the fruit has attracted enormous attention from researchers. A recent study reported that *Punica granatum* seed oil (40 μM), which contains an abundance of unsaturated fatty acid acyl glycerols in the form of diynoic acid derivatives, can confer some protection against 3-NP-induced neurotoxicity in cultured PC-12 cells.[43] The findings indicate suppression of lactase dehydrogenase activity and reduction of ROS, extracellular nitric oxide, and lipid peroxidation occurring in the PC-12 neuronal cells, which are attributed to the antioxidant activity of the seed oil as confirmed by *in vitro* radical scavenging assays. In addition, owing to the presence of the interrupted diynoic acid system by one methylene, the seed oil of *Punica granatum* exerted a superior antioxidant capacity to enhance antioxidant gene expression and neutralize ROS formation in PC-12 cells.[43]

In Vivo Studies. Calendula officinalis Linn. *Calendula officinalis* Linn, also known as marigold, belongs to the family of Asteraceae.[44] From ancient times, the flowers of *Calendula officinalis* have been applied as an herbal medicine for homeopathic purposes due to its ethnomedical value. Phytochemical screening indicates that *Calendula officinalis* flowers are rich in flavonoids (quercetin, isoquercetin, isorhamnetin, calendoflavoside, calendoflaside, calendoflavobioside, and rutin), carotenoids (α-carotene, β-carotene, and α-tocopherol), and quinones (phylloquinone and plastoquinone).[44]

Shivasharan et al.[45] investigated the beneficial effects of *Calendula officinalis* extract in a HD *in vivo* model where constituents such as rutin and chlorogenic and ferulic acids have been identified as the notable bioactive compounds that confer the neuroprotective actions. Interestingly, pretreatment with *Calendula officinalis* (100 and 200 mg/kg, po) markedly alleviated 3-NP-induced striatal oxidative damage, neuronal loss, and behavioral alterations. The observed protective propensity on the neurons is attributed to the antioxidant and anti-inflammatory potential of *Calendula officinalis* which can (1) normalize the endogenous antioxidant defensive enzymes and (2) reduce the oxidative and nitrative stress in the rat brain. Additionally, *Calendula officinalis* has some estrogenic properties, which may prevent the neuronal degeneration due to neurotoxicity since estrogen can increase antiapoptotic and decrease apoptotic gene expression, thus increasing the blood supply to brain regions and inhibiting the expression of pro-inflammatory cytokines.[45]

Celastrus paniculatus Willd. *Celastrus paniculatus* Willd is an Ayurvedic medicinal plant from the Celastraceae family, also locally known as "Tree of life", Kangani, Malkangni, or Jyotishmati.[46] Accumulated reports have indicated that the extracted seed oil from *Celastrus paniculatus* possesses a series of neuroprotective activities together with having good potential as a memory enhancer. For instance, the study by Malik et al.[47] indicated that the seed oil from *Celastrus paniculatus* dose-dependently attenuated behavioral alterations and oxidative damage induced by 3-NP in the rat brain, which effects are largely attributed to its antioxidant activity that reduces both malondialdehyde (MDA) and nitrite levels. The levels of GSH, SOD, and catalase (CAT) activity are also restored in 3-NP intoxicated rats following treatment with *Celastrus paniculatus* (100 mg/kg, po). Additionally, a report by Godkar et al.[48] revealed that *Celastrus paniculatus* aqueous extract and seeds can protect neurons from glutamate-induced neurotoxicity by acting on NDMA receptors culminating in increased calcium (Ca^{2+}) influxes.

Centella asiatica (Linn) Urban. *Centella asiatica* (Linn) Urban, from the Umbelliferae family, is a native plant from India, Madagascar, Malaysia, Sri Lanka, and South Africa. For centuries, it has been widely used in Ayurvedic medicine to treat various ailments. The leaves of *Centella asiatica* contain triterpene saponins (sapogenins), madecassoside (madecassic acid), asiaticoside (asiatic acid), flavonols, and derivatives of caffeic acid, which are potent antioxidants. The various neuropharmacological potentials of *Centella asiatica* (5 mg/kg, po) have been investigated in an HD *in vivo* model.[49,50] In the study, prophylactic treatment using *Centella asiatica* extract conferred remarkable protection against 3-NP-induced protein and neuronal oxidative damage in all regions in the rat brain as evidenced by (1) attenuation of GSH, (2) total thiol (TSH) depletion, and (3) increased protein carbonyl levels. Additionally, *Centella asiatica* can prevent (1) membrane damage and (2) mitochondrial swelling and dysfunction, all of which contribute to the preservation of the metabolic rate in the mitochondria as well as maintenance of ETC integrity. Overall, these protective efficacies are ascribed to the ability of *Centella asiatica* to enhance the endogenous antioxidant status via the attenuation of oxidative stress and elevation of certain enzymatic and nonenzymatic antioxidant levels.

Convolvulus pluricaulis Choisy. In the past decades, *Convolvulus pluricaulis* Choisy. (Convolvulaceae), also known as Shankhpushpi, has been tested in the treatment of various central nervous system disorders.[51] *Convolvulus pluricaulis* is reported to contain alkaloids, flavonoids (kaempferol derivatives), carbohydrates, volatile oil, phytosterol (β-sitosterol),

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scopoletin, 20-oxodotriacontanol, 29-oxodotriacontanol, and tetratriacontanoic acid used as an Ayurvedic nerve tonic called Medhya Rasayana.[52] The tonic is believed to have antianxiety, antidepressant, anticonvulsant, memory enhancing, and sedative effects, useful in relieving insomnia, dyspepsia, fatigue or weakness, nervousness, and palpitation.[53]

Various fractions of *Convolvulus pluricaulis* have been shown to confer some neuroprotective effects as confirmed in 3-NP intoxicated rat models. For example, Kaur et al.[54] indicated that pretreatment with methanolic extracts of *Convolvulus pluricaulis* (20 mg/kg, po) markedly ameliorated 3-NP-induced behavioral changes, which include the restoration of locomotor activity and rotarod and beam-walk performance. Additionally, the oxidative damage in a 3-NP treated rat model was reduced by attenuation of LPO levels and restoration of the defensive enzyme. In another study, Malik et al.[52] reported that the standardized hydromethanol extract of *Convolvulus pluricaulis* (200 mg/kg, po), which contains scopoletin, significantly enhanced memory and cognitive function of 3-NP treated rats, decreasing the time latency (TL) in Morris water maze test (MWM). The findings suggest that the antioxidant activity of *Convolvulus pluricaulis* is exerted in the striatal region of the brain along with the simultaneous upregulation of protein synthesis in the hippocampus.

Ficus religiosa Linn. *Ficus religiosa* Linn (Moreceae), also known as peepal, pipal, or pimpala tree, is a large and deciduous tree with no or few aerial roots that grows in the tropical area throughout India.[55] It is also commonly cultivated in the vicinities of temples in South East Asia since the tree is religiously recognized as a sacred tree to both Buddhist and Hindu communities. The tree is traditionally used in a variety of the medical systems, including the Ayurveda, Homeopathy, Unani, and Siddha. In fact, the therapeutic potential of its various parts including the leaves, fruits, stem barks, aerial roots, seeds, vegetative buds, and latex has been confirmed.[56]

The leaves of *Ficus religiosa* are of the major interest, because they contain lupeol, arginine, aspartic acid, tryptophan, proline, alanine, threonine, α-amyrin, campesterol, isofucosterol, stigmasterol, tyrosine, methionine, isoleucine, leucine, tannic acid, glycine, serine, hexacosanol, *n*-octacosane, *n*-nonacosane, *n*-hentricontanen, and valine contents.[57] These phytochemicals provide a range of pharmacological activities of *Ficus religiosa*, including antioxidant, antiacetylcholinesterase, antidiabetic, anti-inflammatory, analgesic, anticonvulsant, antimicrobial, antiamnestic, antiulcer, and proteolytic activities.[57,58] Bhangale et al.[58] investigated the neuroprotective effect of *Ficus religiosa* at a high dose (400 mg/kg, po) against the 3-NP-induced HD model rats. Pretreatment with the ethanolic and ethyl acetate *Ficus religiosa* extracts significantly mitigated the behavioral, biochemical, and histological alterations purported to be contributed by its high antioxidant properties. The extracts decreased neuronal degeneration and neuroinflammation as well as ameliorated necrosis in the striatum. The tendency toward decreased oxidative stress in the rat brain along with the increased enzymatic and nonenzymatic antioxidant status indicates the neuroprotective properties of *Ficus religiosa*.

Ginseng Radix Rubra. Ginseng radix Rubra is a steamed root of *Panax ginseng*, which is also known as Korean red ginseng.[59] Among several types of *Panax ginseng*, Korean red ginseng has been reported to have useful antidiabetes, antihypertension, antinociception, and anticancer activities. Subsequently, investigation of extract of Korean red ginseng by Jang et al.[60] suggested that preadministration of the extract (50, 100, and 250 mg/kg, po) dose-dependently alleviated neurologic impairment and lethality, accompanied by the decrease in striatal neuronal death and lesion area, followed by striatal degeneration. These protective effects may be contributed by the anti-inflammatory properties of the extract on striatal neurotoxicity. Another observation indicates that microglial activation, pro-inflammatory cytokine expression [interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α)], and elevation of iNOS are inhibited with pretreatment of Korean red ginseng extract, by deactivation of the mitogen-activated protein kinases (MAPK) phosphorylation and nuclear factor kappa light chain enhancer of activated B cells (NF-κB) signaling pathways. Hence, ginseng radix warrants attention as a potential medicinal plant extract in HD *in vivo* model.

Luehea divaricata Mart. *Luehea divaricata* Mart (Tiliaceae) is a well-known natural product found in South America in the Southern regions in Brazil and also known as acoita-cavalo by the natives.[61] Its leaves are reported to be rich in flavonoids, saponins, mucilage, and tannins, while the crude extract contains alkaloids, anthocyanidins, polysaccharides, carotenoids, and some fixed oils.[62] For these reasons, *Luehea divaricate* has been claimed to have anti-inflammatory and antimicrobial activities and is primarily used to treat rheumatism, arthritis, blennorrhea, dysentery, leukorrhea, skin lesions, and any other gastrointestinal and respiratory infections.[61,62] Courtes et al.[63] investigated the neuroprotective action of *Luehea divaricata* extract against HD-associated behavioral and biochemical changes as induced by 3-NP in the rat model. Interestingly, *Luehea divaricata* aqueous extract (500 and 1000 mg/kg, ig) tends to normalize the behavioral and motor deficits, improving the rotarod and locomotor performances. In addition, the biochemical alteration was attenuated corresponding to decreased oxidative stress and lipid peroxidation in the striatum and cortex, indicating the protective effect of this plant extract against HD *in vivo* model ameliorated via its strong antioxidant activity.

Panax quinquefolius Linn. *Panax quinquefolius* Linn, which is in the Araliaceae family, is also known as American ginseng. In the past decades, there have been many clinical studies worldwide supporting the benefits of *Panax quinquefolius* notably in neurodegenerative disease.[64] Enormous efforts have also been devoted to the investigation on ginsenosides, major active components of ginseng extract especially with regards to the stems and leaves, which have favorable pharmacological activities, including antioxidant, antineoplastic, and antistress properties.[65] For example, ginsenosides, isolated from ginseng extract, consist of a steroid-like four ring structure attached to sugar moieties[66] and are the major components with putative neuroprotective potencies.

Interestingly, American and Asian ginsengs contain unique relative amounts of ginsenoside groups, panaxadiols (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, and Rh3) and panaxatriols (Re, Rf, Rg1, Rg2, and Rh1).[64] As reported by Lian et al.,[65] the isolated Rb1 (panaxadiol, 10 mg/kg, po) elicits putative neuroprotective activities, which included reduced striatal lesion volume and hippocampal neuron loss in 3-NP administered rats. In addition, Rb1 also ameliorates the behavioral changes and motor impairment in the HD *in vivo* model, suggesting its potent neuronal protection by virtue of its free radical scavenging properties.

Withania somnifera (Linn) Dunal. *Withania somnifera* (Linn) Dunal, from the Solanaceae family, is an important

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Table 4. List of Natural Products (Isolated Compounds) with Potential against HD in Experimental Models

natural compounds	type of natural compounds	sources	refs
berberine	alkaloids	*Coptis chinensis*, *Berberis* species	Jiang et al.[32]
celastrol	triterpene	*Tripterygium wilfordii*	Cleren et al.[80]
dihydromyricetin	flavonoid	*Ampelopsis grossedentata*	Mu et al.[85]
embelin	*para*-benzoquinone	*Embelia ribes*	Dhadde et al.[88]
epigallocatechin gallate (ECGC)	polyphenol	*Camellia sinensis*	Kumar and Kumar[90]
esculetin		*Foeniculum vulgare*, *Aesculus hippocastanum*, *Salvia officinalis*	Karandikar and Thangarajan[92]
forskolin	diterpenoid	*Coleus forskohlii*	Mehan et al.[94]
genistein	isoflavone	soybeans	Menze et al.[97]
gintonin	lysophosphatidic acid	ginseng	Jang et al.[139]
lutein	carotenoid	green leafy vegetables, carrots	Binawade and Jagtap[100]
lycopene	carotenoid	tomatoes and tomato products	Sandhir et al.[17]
naringin	flavonoid	citrus fruits	Gopinath and Sudhandiran[141]
neferine	alkaloid	*Nelumbo nucifera*	Wong et al.[74]
nicotine		*Nicotiana tabacum*	Tariq et al.[101]
onjisaponin B		*Radix polygalae*	Wu et al.[77]
praeruptorin C		*Peucedanum praeruptorum*	Wang et al.[107]
protopanaxatriol	ginsenosides	*Panax ginseng*	Gao et al.[143]
puerarin	isoflavonoid	*Radix puerariae*	Mahdy et al.[112]
quercetin	flavonoid	fruits and vegetables	Chakraborty et al.[116]
resveratrol	phytoalexin	grapes	Kumar et al.[118]
S-allylcysteine	organosulfur compound	aged garlic	Elinos-Calderón et al.[120]
sesamol	phenolic compound	*Sesamum indicum*	Kumar et al.[124]
solanesol	polyisoprenoid alcohol	*Nicotiana tabacum*	Mehan et al.[126]
spermidine	polyamine	green vegetables, meat and milk products	Jamwal and Kumar[128]
sulforaphane	isothiocyanate	cruciferous vegetables	Jang and Cho[145]
T1-11		*Gastrodia elata*	Huang et al.[147]
tetramethylpyrazine	heterocyclic compound	*Ligusticum wallichii*	Danduga et al.[130]
trans-(−)-ϵ-viniferin	stilbenoid	*Vitis* spp. (wild grape)	Fu et al.[78]
α-mangostin	xanthone	*Garcinia mangostana*	Pedraza-Chaverrí et al.[79]
L-theanine	amino acid	*Camellia sinensis*	Thangarajan et al.[134]

medicinal plant. It has high value as an anabolic agent and is said to be health restorative, extensively applied in the Ayurvedic medicine system.[67] *Withania somnifera* is well-known for its valuable therapeutic effects of anti-inflammatory, antimicrobial, antiarthritic, antistress, antidepressant, cardioprotective, and neuroprotective activities.[68] These biological activities are contributed by pharmacologically active phytochemicals, glycowithanolides, responsible for its protective effects against various diseases. *Withania somnifera* root extract (100 and 200 mg/kg, po) significantly improved motor coordination and increased grip strength of 3-NP treated rats in a dose-dependent manner.[69] The effect may be attributed to the restoration of the antioxidant enzyme level and the mitochondrial enzyme complex activity, indicating a possible antioxidant role of *Withania somnifera* against 3-NP-induced neurotoxicity in a HD *in vivo* model. Additionally, it has been postulated that *Withania somnifera* has the possible anabolic effect of reducing the 3-NP triggered ATP loss and energy impairment. It is plausible that the protective activity directly restores the cellular energetic balance and, in turn, prevents mitochondrial damage due to 3-NP-induced toxicity.

Zingiber officinale Roscoe. In the past 2000 years, *Zingiber officinale* Roscoe also known as ginger, has been utilized as a spice in food preparations around the world.[70] Traditionally, it has been applied as the main ingredient in Ayurvedic, Tibbi-Unani herbal, and Chinese medicine systems for treatment of asthma, rheumatism, gingivitis, hypertension, stroke, diabetes, and constipation.[71] The ginger root and its aqueous extract possess certain polyphenol compounds, such as 6-gingerol and its derivatives,[72] suggesting the possible antioxidant properties of the plant to be a neuroprotectant against neurodegenerative disease. Sharma et al.[71] confirmed the protective action of ginger root extracts in the 3-NP-induced HD animal model in which chronic treatment with ginger ethanolic extract (100 and 200 mg/kg, po) dose-dependently (1) improved memory, (2) mitigated motor and cognitive dysfunction, and (3) restored the biochemical changes with increased GSH and decreased nitrosative and oxidative stress levels in 3-NP treated rats. Taken together, the findings provide a new possibility of *Zingiber officinale*, which exerts its neuroprotective action via anticholinesterase potency.

Protective Effects of Natural Products (Isolated Compounds) against HD. The list of isolated natural compounds investigated as alternative medicines in HD preclinical models is outlined in Table 4, with their chemical structures depicted in Figure 5. Comprehensive information on their neuroprotective efficacy against HD from *in vitro* and *in vivo* studies are summarized in Tables 5 and 6, respectively.

In Vitro Studies. Neferine. Neferine is a bisbenzylisoquinoline alkaloid isolated from the seed embryo of *Nelumbo nucifera* Gaertn (Nelumbonaceae). *Nelumbo nucifera*, also known as lotus, is an edible and traditional Chinese medicinal plant that was initially cultivated in China.[73] Based on the report from Shenong from the Liang Dynasty, all of the plant parts (including the fruits, seeds, and roots) have a variety of medicinal effects and are commonly applied as antiaging and anxiety relief.[37] Due to its nontoxic nature, it is also widely used as a main ingredient in soup and tea preparations in

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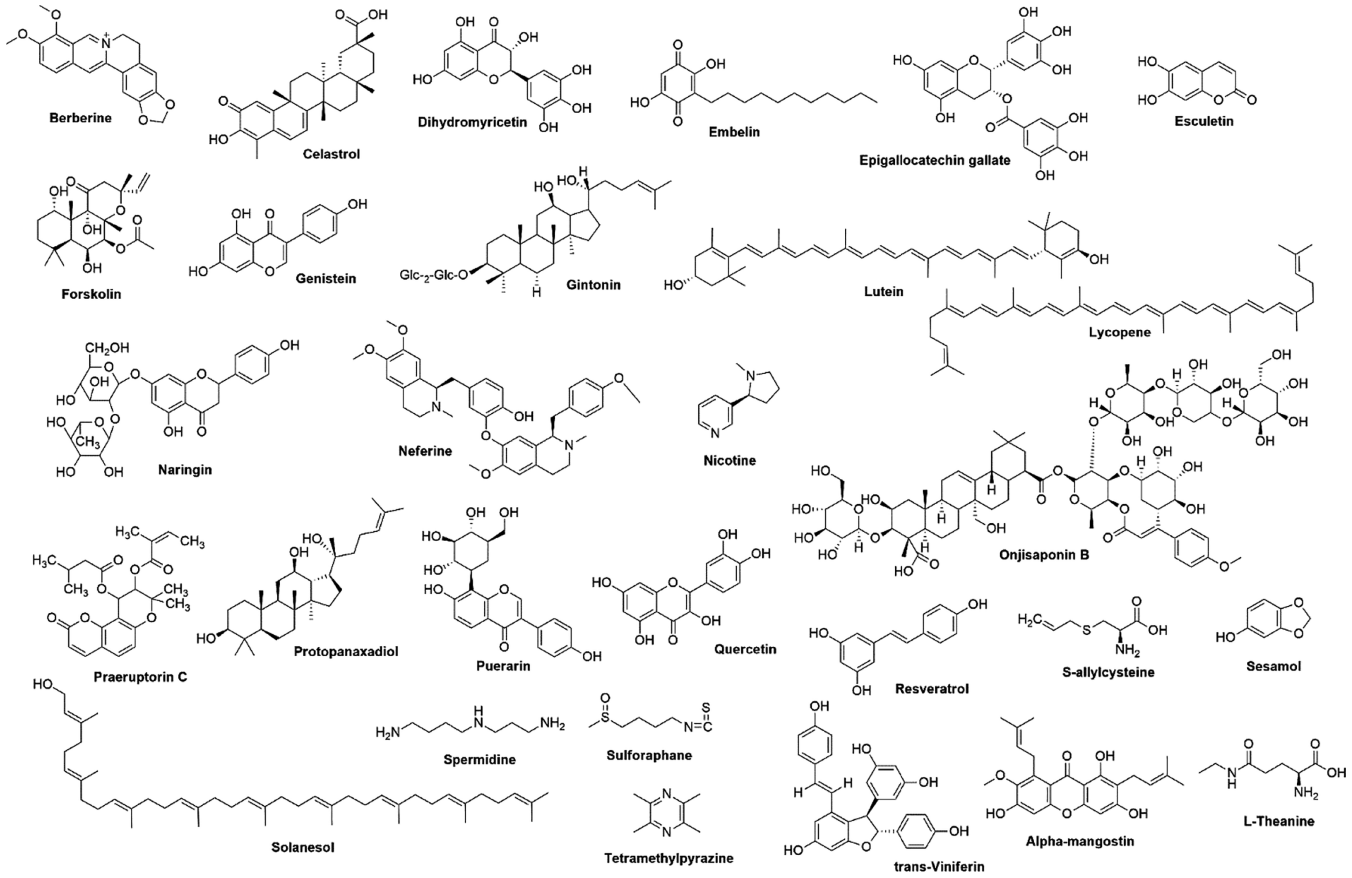

Figure 5. Chemical structure of naturally isolated compounds against HD in experimental models.

China. Neferine has potential as a neuroprotective agent against mHtt in PC-12 cells.[74] The data supports a working model for neferine (7.5 μM) decreasing 3-NP-induced toxicity via induction of mammalian target of rapamycin (mTOR)−AMP-activated protein kinase (AMPK) [mTOR−AMPK] dependent autophagy by its activity on the autophagy related gene 7 (*Atg7*) with a higher expression of *Htt*.

Onjisaponin B. Onjisaponin B is one of the bioactive compounds that can be identified in radix polygalae (Yuan Zhi), the dried root of *Polygala tenuifolia* Willd, which is a popular Chinese medicinal plant traditionally used to promote tranquilization and mental alertness in China.[75] *Radix polygalae* extracts are reported to contribute to antipsychotic, antidepressant, memory, cognitive improvement, and sleep-promoting effects.[76] Therefore, to date, *Radix polygalae* extract is used to modulate neurodegenerative disorders and insomnia. Onjisaponin B was predominantly highlighted by Wu et al.[77] due to its neuroprotective potential in the HD *in vitro* model. Accordingly, *Radix polygalae* ethanolic extract markedly increased the conversion of LC3-I to LC3-II in PC-12 cells. Based on protein expression, the rate of LC3-II formation increased concomitantly with the existence of protease inhibitors, suggesting that onjisaponin B (25 and 50 μM) inhibited neuronal damage via autophagy, which was activated due to the gene regulation of *Atg7* via the mTOR−AMPK signaling pathway. The discovery successfully confirms that onjisaponin B has the potential to be a novel and effective autophagy enhancer without significant cytotoxicity, thus promoting the removal of mHtt and α-synuclein in PC-12 neuronal cells.

trans-(−)-ϵ-Viniferin. *trans*-(−)-ϵ-Viniferin is a unique collection of 22 types of stilbenic compounds composed of natural resveratrol monomers and oligomers. Fu et al.[78] reported that *trans*-(−)-ϵ-viniferin (EC_{50} = 30 nM to 10 μM) can protect cells from the detrimental effects of HD in an *in vivo* model. The neuroprotective effects are mainly exerted via the mediation of sirtuin-3 (SIRT3), a soluble protein responsible for controlling mitochondrial protein acetylation. In HD pathophysiology, mHtt induces the direct depletion of mitochondrial SIRT-3. In contrast, viniferin increases the levels of both SIRT-3 isoforms and activates the deacetylase activity of SIRT-3 in targeted substrate Mn-SOD, ultimately leading to enhancement of putative antioxidant activities. Subsequently, SIRT-3 deacetylates liver kinase B1 (LKB1) and directly induces AMPK activation, resulting in an increase of mitochondrial biogenesis that is accompanied by elevation of energy metabolism, which prevents the occurrence of mHtt induced mitochondrial dysfunction.[78] Additionally, activated AMPK also (1) replenishes the levels of cellular nicotinamide adenine dinucleotide (NAD^+) and (2) repetitively induces the activation of SIRT-3 and energy metabolism in order to protect the cells from oxidative damage.

α-Mangostin. α-Mangostin is a yellowish xanthone isolated compound from dried sap and bark of *Garcinia mangostana* (Guttiferae) is a tropical tree commonly cultivated in Malaysia, Indonesia, Thailand, Philippines, and Sri Lanka.[79] The pericarp of *Garcinia mangostana* has been widely used to treat diarrhea, abdominal pain, infectious wounds, chronic ulcers, and dysentery. α-Mangostin is claimed to have neuropharmacological activities against 3-NP-induced cultured cerebellar

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Table 5. Summary of Natural Products (Isolated Compounds) with Potential against HD Based on *In Vitro* Studies[a]

natural compounds	dose	IC_{50}	EC_{50}	cell lines	model	molecular outcomes	mode of action	refs
berberine	5, 15, 50[b], 100 μM	*d*	*d*	HEK293 cells	genetically modified with Htt-120Q	Htt aggregation ↓, LC3-I/LC3-II ratio ↓	induction of autophagy	Jiang et al.[32]
gintonin	0.1, 1, 10 μg/mL[c]	*d*	*d*	STHI*dh* cells	genetically modified with wild-type (STHI$dh^{Q7/Q7}$) and mutant (STHI$dh^{Q111/Q117}$) cells	mHtt ↓; neuronal death ↓	inhibition of apoptosis	Jang et al.[139]
naringin	10 μM	*d*	*d*	PC-12 cells	3-NP	neurotoxicity ↓, LDH activity ↓; SOD ↑, CAT activity ↑, GPx ↑, GR ↑, GSH ↑; LPO ↑, ROS ↑; mitochondrial dysfunction ↓; activation of Nrf2 ↑	Nrf2 activation	Kulasekaran and Ganapasam[18]
neferine	7.5 μM	12.8 μM	*d*	cultured *Atg7* and *Atg7* deficient cells (WT-MEF); PC-12 cells	genetically modified	LC3-II ↑; mHtt ↓, cell death ↓	induction of mTOR−AMPK-dependent autophagy	Wong et al.[74]
onjisaponin B	25, 50 μM	*d*	*d*	cultured *Atg7* and *Atg7* deficient cells (WT-MEF); PC-12 cells	genetically modified	LC3-I to LC3-II conversion ↑; *Atg7* gene dependent; AMPK−mTOR inhibited	clearance of mHtt in PC-12 cells via autophagy induction	Wu et al.[77]
protopanaxatriol	0.01[b], 0.1[b], 1.0 μM	*d*	*d*	cultured MSN	glutamate	NMDA-induced Ca^{2+} response ↓	inhibition of Ca^{2+} signaling	Wu et al.[66]
sulforaphane	2, 4, 8 μM[c]	*d*	*d*	HeLa and HEK293 cells	genetically modified with GFPu (GFP fused with degron CL1)	GFPu ↓, mHtt ↓, neurotoxicity ↓	activation of UPS and Atg pathways	Liu et al.[144]
T1-11		4.66 μM	~2.2 μM	PC-12 cells with mHtt	genetically modified	mHtt aggregation ↓, proteasome activity ↑	induction of $A_{2A}R$ signaling and PKA-dependent pathway	Huang et al.[146]
trans-(−)-ϵ-viniferin		*d*	30 nM to 10 μM	immortalized striatal precursor cells, Tet-Off PC-12 cells, N2a cells, cultured primary cortical neurons	genetically modified with mHtt (N63-148Q)	ROS ↓, NAD^+/NADH ratio ↑, mitochondrial biogenesis ↑, SIRT3 and deacetylase activity ↑, activated AMPK ↓	activation of AMPK via by increasing SIRT3	Fu et al.[78]
α-mangostin	0−20 μM	5, 6, 8 μM	*d*	cultured CGNs	3-NP	($^1O_2$) ↓, $O_2^{\bullet-}$ ↓, $ONOO^-$↓, cell damage ↓	scavenge ROS	Pedraza-Chaverri et al.[79]

[a]Abbreviations: 3-NP, 3-nitropropionic acid; Atg, autophagy; CAT, catalase; GFP, green fluorescent protein; GFPu, GFP-UPS reporter; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; Htt, huntingtin; JNK, c-Jun N-terminal kinase; LC3-I, cytosolic form of LC3; LC3-II, LC3-phosphatidylethanolamine conjugate; LDH, lactate dehydrogenase; LPO, lipid peroxidation; mHtt, mutant huntingtin; N2a, neuro-2A; Nrf2, nuclear factor erythroid 2-related factor 2; NAD, nicotinamide adenine dinucleotide; NADH, NAD + hydrogen (H); PC-12, pheochromocytoma-12; ROS, reactive oxygen species; SIRT3, sirtuin-3; SOD, superoxide dismutase; UPS, ubiquitin proteasome system. [b]Most effective concentration. [c]Concentration dependent [d]Not determined.

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Table 6. Summary of Natural Products (Isolated Compounds) with Potential against HD Based on *In Vivo* Studies[a]

natural compounds	dose (mg/kg), route	duration (days)	animal models, sex	toxin	outcomes: behavioral	outcomes: biochemical	outcomes: histopathological	mode of action	refs
berberine	40, po		transgenic mouse: N171-82Q mice, male		motor coordination ↑, muscle strength ↑, body balance ↑	Htt aggregation ↓; p62 ↓		induction of autophagy	Jiang et al.[32]
celastrol	3, ip	10	Lewis rats, male	3-NP, ip		Hsp70 expression ↑, astrogliosis ↓	striatal lesion volume ↓	anti-inflammatory protection by inducing Hsp70	Cleren et al.[80]
dihydromyricetin	10, ip	5	SD rats, male	3-NP, ip	body balance ↑, ELT ↓, TSTQ ↑	striatal glucose metabolism ↑; MDA ↓; SOD ↑	striatal injury ↓	antioxidant and antiapoptotic protection	Mu et al.[85]
embelin	10, 20; po	14	Wistar rats	3-NP, ip	motor abnormalities ↓, locomotor count ↑, memory loss ↓, body balance ↑, hanging latency ↑	LPO ↓, GSH ↑, CAT activity ↑, GST ↑	brain lesions ↓	antioxidant defense system	Dhadde et al.[88]
epigallocatechin gallate (ECGC)	10, 20, 40; po	14	Wistar rats, male	3-NP, ip	locomotor activity ↑, grip strength ↑	MDA ↓, NIT ↓, SOD ↑, CAT activity ↑; NADH, SDH, cytochrome oxidase activities ↑; viable cells ↑	striatal lesion volume ↓	NOS inhibition	Kumar and Kumar[90]
esculetin	25[b], 50; po	14	Wistar rats, male	3-NP, ip	mobility in OFT ↑, TL ↓, immobility times in FST ↓, grip strength ↑	MDA ↓, protein carbonyl ↓; SOD ↑, CAT activity ↑, GPx ↑; SDH activity ↑; AChE ↓		scavenging free radicals	Karandikar and Thangarajan[92]
forskolin	10, 20, 30[b]; ig	15	Wistar rats, male	3-NP, ip	TL ↓, TSTQ ↑, locomotor count ↑, grip strength ↑, neurological score ↓	ATP ↑, SDH activity ↑, LDH activity ↓; AChE ↓; MDA ↓, NIT ↓; GSH ↑, SOD ↑, CAT activity ↑	histological alteration by 3-NP ↓	activation of AC via enhancing cAMP/PKA/CREB pathway	Mehan et al.[94]
genistein	5, 10, 20[b]; ip	8	albino rats, female	3-NP, ip	locomotor activity ↑, memory loss ↓	ATP ↑; LPO ↓, GSH ↑, CAT activity ↑, AchE ↓; iNOS ↓, COX-2 ↓	brain histological features restored	antioxidant and antiapoptotic protection	Menze et al.[97]
gintonin	25, 50, 100[b]; po		C57BL/6NTac mice, male	3-NP, ip; AAV vector	neurological dysfunction ↓	SDH activity ↑; TNF-α, IL-1β, IL-6, COX-2 and iNOS ↓; apoptosis ↓; activation of NF-κB ↓ microglial activation ↓	striatal cell death ↓	activation of LPA and Keap1−Nrf2−ARE pathway, inhibition of MAPKs and NF-κB	Jang et al.[139]
lutein	50, 100;[c] po	14	SD rats, female	3-NP, ip	TL ↓, ELT ↓, locomotor activity ↑	NADH, SDH activities ↑, MTT ↑; GSH ↑, CAT activity ↑, LPO ↓; AchE ↓	mild focal gliosis	scavenging free radicals	Binawade and Jagtap[100]
lycopene	10; po	15	Wistar rats, female	3-NP, ip	body balance ↑, TL ↓, memory loss ↓	NADH, SDH, cytochrome oxidase, F_1F_0 synthase activities ↑; NIT ↓, ROS ↓, LPO ↓; SOD ↑; TSH ↑, PSH ↑, NPSH ↑; mitochondrial cytochrome *c* ↑, p53 expression ↓		↓ oxidative stress, ↓ mitochondrial dysfunction	Sandhir et al.[17]
naringin	80; po	14	Wistar rats, male	3-NP, ip	hind limb function ↑, grip strength ↑, print length ↓	mMP ↑, TIMP ↑; NF-κB ↓, GFAP ↓		modulation via expression of MMPs and GFAP	Gopinath and Sudhandiran[141]
						GSH/GSSG ratio ↑; activation of Nrf2 ↑; TNF-α, COX-2 and iNOS ↓	histopathological alterations ↓	activation of Nrf2-mediated ARE gene pathway	Gopinath and Sudhandiran[142]
						SOD ↑, CAT activity ↑, GPx ↑, GR ↑; GSH ↑, vitamin C and E ↑; ATPases ↑; Bax ↓, Bad ↓, Bcl-2 ↑; mitochondrial cytochrome *c* ↑, cytosolic caspase-3 activation ↓	histopathological abnormalities in striatum ↓	antioxidant and antiapoptotic protection	Gopinath et al.[140]
nicotine	0.25, 0.50, 1.0;[c] ip	7	Wistar rats, female	3-NP, ip	locomotor activity ↑, grip strength ↑, body balance ↑, higher capacity angles in inclined plane test	DA ↑; GSH ↑; TH ↑	striatal lesions ↓	restoration of striatal DA	Tariq et al.[101]
	1.5; ip	8	Wistar rats, male	3-NP, ip		LPO ↓, protein carbonyl ↓, SDH activity ↑		antioxidant defense system	Túnez et al.[104]

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Table 6. continued

natural compounds	dose (mg/kg), route	duration (days)	animal models, sex	toxin	outcomes: behavioral	outcomes: biochemical	outcomes: histopathological	mode of action	refs
praeruptorin C	1.5, 3.0; po	8	C57BL/6 mice	3-NP, ip	fall latency in rotarod ↑, total distance in OFT ↑, immobility times in TST and FST ↓	DARPP-32 ↑, BDNF ↑; glutamate ↓	neuronal damage in the striatum ↓	↑ expression of normal Htt, DARPP-32, and BDNF	Wang et al.[107]
protopanaxatriol	5, 10[b], 20; po	5	SD rats, male	3-NP, ip	locomotor activity ↑	ROS ↓; SDH activity ↑, SOD ↑; Hsp70 expression ↑; Nrf2 activation ↑, HO1 and NQO1 expression ↑	normal morphology of nucleus, number of cells decreased in the striatum ↓	↑ antioxidant defense system	Gao et al.[143]
puerarin	200; ip	5	albino Wistar rats, male	3-NP, sc		cytosolic caspase-3 and cytochrome *c* ↓, Bax ↓, Bcl-2 ↑, ATP ↑, TNF-α ↓, iNOS ↓, NF-κB ↓		antiapoptotic and anti-inflammatory protection	Mahdy et al.[112]
quercetin	25, 50; ip	4	SD rats, male	3-NP, ip	anxiety ↓, motor coordination ↑, gait abnormalities ↓	serotonin metabolism ↓, MAO-A ↓	microglial proliferation ↓, astrocytes numbers ↑	scavenging free radicals, anti-inflammation protection	Chakraborty et al.[116]
resveratrol	5, 10;[c] po	12	Wistar rats, male	3-NP, ip	TL ↓, VCM ↓, locomotor activity ↑	GSH ↑; MDA ↓, NIT ↓; SDH activity ↑		antioxidant defense system, scavenging free radicals	Kumar et al.[118]
S-allylcysteine	300; ip		Wistar rats, male	3-NP, ip		LPO ↓, SOD ↑; MTT reduction ability ↑		↓ oxidative stress	Herrera-Mundo et al.[121]
sesamol	5, 10, 20; po	14	Wistar rats, male	3-NP, ip	cognitive and memory performance ↑	ratio of GSH/GSSG ↑, GST ↑, LDH activity ↓		scavenging free radicals	Kumar et al.[123]
	5, 10, 20; po	14	Wistar rats, male	3-NP, ip	locomotor activity ↑, muscle strength ↑	MDA ↓, NIT ↓, SOD ↑, CAT activity ↑, NADH, SDH, cytochrome oxidase activities ↑; viable cells ↑		reverse mitochondrial enzyme status	Kumar et al.[124]
solanesol	5, 10, 15;[c] po	15	Wistar rats, male	3-NP, ip	ELT ↓, TSTQ ↑, grip strength ↑, TL ↓, locomotor count ↑, balance beam-walking improved	ATP ↑, SDH activity ↑, GSH ↑, SOD ↑, CAT activity ↑; AChE ↓; MDA ↓, NIT ↓	rat brain optimally sized, cell nucleus and continual cell membrane observable	↓ mitochondrial dysfunction	Mehan et al.[126]
spermidine	5, 10;[c] po	21	Wistar rats, male	3-NP, ip	locomotor activity and motor coordination ↑, TL ↓	LPO ↓, NIT ↓; total NPSH ↑; expression of TNF-α, IL-1β, IL-6 ↓; catecholamine ↓; GABA ↓, glutamate ↓; purine ↓		antioxidant and anti-inflammatory protection	Jamwal and Kumar[128]
sulforaphane	2.5, 5.0[b]; ip	8	C57BL/6 mice, male	3-NP, ip	neurological dysfunction ↓	SDH activity ↑; TNF-α, IL-1β, IL-6, COX-2 and iNOS ↓; apoptosis ↓; activation of JNK, ERK and NF-κB ↓ microglial activation ↓; Nrf2 ↑	striatal lesions ↓	activation of Keap1−Nrf2−ARE pathway, inhibition of MAPKs and NF-κB	Jang and Cho[145]
			transgenic mouse with GFPu			GFPu ↓, UPS function ↑; Atg activity ↑		activation of UPS and Atg pathways	Liu et al.[144]
tetramethylpyrazine	40, 80;[c] ip	21	Wistar rats, male	3-NP, ip	TL ↓, spatial memory impairment ↓, locomotor count ↑, motor coordination ↑	SDH activity ↑, GSH ↑, SOD ↑, CAT activity ↑; GABA ↑, glutamate ↓; AChE ↓; MDA ↓	histological changes in striatum ↓, neuronal count in the hippocampus ↑	↑ antioxidant, ↓ lipid peroxidation	Danduga et al.[130]
T1-11	0.05; ip		transgenic R6/2 mice, male		deterioration of motor coordination ↓	activation of $A_{2A}R$ ↑, adenosine uptake ↓, cAMP ↑, striatal mHtt ↓, proteasome activity ↑		activation of the adenosinergic system	Huang et al.[147]
L-theanine	100, 200;[c] po	14	Wistar rats, male	3-NP, ip	TL ↓, grip strength ↑, mobility ↑	LPO ↓, GSH ↑; SOD ↑, CAT activity ↑; SDH activity ↑	mild neuronal cell damage, reduced inflammation, absence of necrosis and gliosis	antioxidant defense system	Thangarajan et al.[134]

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granule neurons (CGNs). The neuroprotective potential of α-mangostin against 3-NP triggered neurotoxicity was closely linked to the attenuation of peroxynitrite anion (IC_{50} = 5 μM), superoxide anion (IC_{50} = 6 μM), and singlet oxygen (IC_{50} = 8 μM) generation in cultured CGNs,[79] thus suggesting that the scavenging of ROS is contributed by its antioxidant and anti-inflammatory activities.

In Vivo Studies. Celastrol. *Tripterygium wilfordii* Hook (Celastraceae) is an ivy-like vine with a long story of use in Chinese traditional medicine to treat joint pain and fever. The root bark extract of *Tripterygium wilfordii* contains celastrol, a triterpene with known putative neuropharmacological effects against 3-NP-induced neurotoxicity in the HD animal model. In rats administered 3-NP, celastrol (3 mg/kg, ip) was demonstrated to suppress the production of pro-inflammatory cytokines and iNOS and microglial activation.[80] Additionally, it inhibits LPO in the inner and outer mitochondrial membrane by a direct free radical scavenging activity. Moreover, celastrol can induce the activation of DNA-binding activity of heat shock factor 1 (HSF1), followed by a higher expression of Hsp70 in the lateral brain striatum.[81] Therefore, it was concluded that celastrol can reduce 3-NP-triggered astrogliosis markedly in the rat brain. Subsequently, it was confirmed that celastrol conferred complete protection against striatal lesions and neurotoxicity induced by 3-NP via its antioxidant and anti-inflammatory abilities.

Dihydromyricetin. Dihydromyricetin is an isolated flavonoid abundantly found in *Ampelopsis grossedentata*, commonly known as vine tea (Tengcha).[82] In Asia, vine tea has been traditionally used as a medicinal herbal tea and was believed to have health benefits in preventing and treating sore throat, common flu, jaundice hepatitis, and hypertension.[83] There are studies revealing the promising pharmacological properties of dihydromyricetin, including antioxidant, anti-inflammatory, hepatoprotective, anti-membrane lipid peroxidation, antithrombotic, anticarcinogenic, and antibacterial activities.[82,84] The neuroprotective effects of dihydromyricetin have been demonstrated in a HD *in vivo* model involving 3-NP induced motor and cognitive deficits and striatal injury.[85] Dihydromyricetin (10 mg/kg, ip) improves energy metabolism in the striatum by decreasing MDA and increasing SOD level via the antioxidant defense system. Apart from this, dihydromyricetin has been shown to reverse 3-NP induced up-regulation of Bax and down-regulation of Bcl-2. Also, 3-NP induced cell apoptosis was markedly reduced in the dihydromyricetin treated groups. These findings indicate that dihydromyricetin offers mitigation of motor and cognitive impairments as well as striatal injury by virtue of antioxidant and antiapoptotic activities.

Embelin. *Embelia ribes* Burm belongs to the Myrsinaceae family. It is a natural product documented in Ayurvedic medicine from ancient times,[86] extensively used as a major ingredient in various Indian formulations. Embelin, chemically known as 2,5-dihydroxy-3-undecyl-1,4-benzoquinone, is the main active constituent from all the plant parts of *Embelia ribes*. It is a naturally occurring alkyl-substituted hydroxybenzoquinone.[87] Considerable evidence suggests that embelin possesses antioxidant, anticonvulsant, analgesic, anti-inflammatory, and antidiabetic properties.[86,88] Additionally, emerging research on embelin has established its promising neuroprotective potential in a 3-NP-induced neurotoxicity rat model. Dhadde et al.[88] revealed that the administration of embelin (10 and 20 mg/kg, po) can alleviate the damage to striatal neurons from 3-NP,

Table 6. continued

[a]Abbreviations: 3-NP, 3-nitropropionic acid; AAV, adeno-associated virus; AchE, acetylcholinesterase; ARE, antioxidant response element; Atg, autophagy; ATP, adenosine triphosphate; Bad, Bcl-2-associated agonist of cell death; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CAT, catalase; COX-2, cycloxygenase-2; ELT, escape latency; ERK, extracellular signal-regulated kinase; FST, forced swimming test; GABA, γ-aminobuytric acid; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; GFPu, GFP-UPS reporter; GR, glutathione reductase; GSH, reduced glutathione; Htt, huntingtin; ip, intraperitoneal injection; IL-1β, interleukin-1β; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; LPA, lysophosphatidic acid; LPO, lipid peroxidation; MAO-A, monoamine oxidase A; MDA, malondialdehyde; mMP, mitochondrial membrane potential; MMP, matrix metalloproteinase; NAD, nicotinamide adenine dinucleotide; NADH, NAD + hydrogen (H); NIT, nitrite; NPSH, non-protein thiols; Nrf2, nuclear factor erythroid 2-related factor 2; OFT, open field test; po, per os, oral administration; p53, tumor protein 53; p62, nucleoporin 62; PSH, protein thiols; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TIMP, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor α; TSH, total thiols; UPS, ubiquitin proteasome system; VCM, vacuous chewing movement. [b]Most effective dose. [c]Dose dependent.

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which in turn confers protection from the deleterious effects seen in behavioral and neurochemical alteration. The neuroprotective effect of embelin is attributed to (1) its free radical scavenging properties, (2) decreased lipid peroxide levels, and (3) increased nonenzymatic and enzymatic antioxidant levels. By normalizing the altered antioxidant defense system, embelin administration provides a possible mode of neuroprotection in animals with HD-like symptoms.

Epigallocatechin Gallate (ECGC). *Camellia sinensis* (Linn) is also known as green tea and is said to have originated from Southwest China. The young or mature leaves, stems, and shoots of *Camellia sinensis* are used to process different types of green tea products.[89] Owing to its attractive pharmacological effects, including anticarcinogenic, antimutagenic, antioxidant, antiproliferative, and neuroprotective properties,[90] green tea is the most popular herb used to make tea beverages all around the world. Green tea leaf extracts, L-theanine and epigallocatechin gallate (ECGC), have been confirmed to be effective in neuroprotection against 3-NP-induced toxicity of HD experimental model.

Epigallocatechin gallate (ECGC) is an important polyphenol present in green tea that can pass the blood−brain barrier (BBB). According to Kumar and Kumar,[90] the putative neuroprotective actions of ECGC (10, 20, and 40 mg/kg, po) involve attenuation of 3-NP-induced behavioral deficits and restoration of mitochondrial complex enzyme activities in order to preserve normal ATP levels. mHtt protein reduction and calcium influx inhibition are considered to be vital mechanisms in preventing neuronal damage. These protective actions can be explained by its antioxidant effects, which involve free radical scavenging, metal ion chelating, and ROS reduction. In addition, nitric oxide mediation may also contribute to the neuroprotective effects of ECGC by inhibiting nNOS and iNOS induction in the HD *in vivo* model.

Esculetin. Esculetin, a natural coumarin, is a secondary metabolite isolated from various plants, including *Salvia officinalis*, *Aesculus hippocastanum*, and *Foeniculum vulgare*.[91] Esculetin has been reported to have anticancer, anti-ischemic, antidiabetic, and neuroprotective properties.[92] Motivated by these pharmacological potentials of esculetin, its beneficial effects in the 3-NP-induced HD *in vivo* model have been investigated, where its most effective dose (25 mg/kg, po) significantly alleviated behavioral and biochemical alterations.[92] In terms of behavioral improvement, the herb enhanced mobility in the open field test (OFT) and forced swimming test (FST), as well as the grip strength, of 3-NP treated rats. Additionally, lipid peroxidation was markedly reduced along with the restoration of antioxidant enzymes, suggesting that the possible neuroprotection occurs via antioxidant and free radical scavenging activity.

Forskolin. *Coleus forskohlii*, which belongs to the mint family, Lamiaceae, is an indigenous medicinal plant that originates from India and is locally known as mayani or makandi in Ayurveda.[93] Its tuberous root extract, forskolin, is the main constituent iterpenoid shown to possess the pharmacological effect on cardiovascular disease, hypertension, psoriasis, asthma, and eczema. Interestingly, intragastric administration of the natural product at 30 mg/kg markedly ameliorated the motor dysfunction and memory impairment of rats administered 3-NP.[94] It is plausible that attenuation of memory impairment mainly involved the brain cholinergic system, in which acetylcholinesterase (AChE) levels were reduced owing to the antioxidant capacity of forskolin in scavenging free radical, followed by an increase in ATP levels. Therefore, based on the above-mentioned observations, the vital role of adenyl cyclase (AC) activation in cAMP/CREB signaling pathway was investigated. Interestingly the results showed that AC/cAMP/PKA/CREB activation was responsible for attenuating HD-like symptoms in 3-NP treated rats.[95]

Genistein. Genistein, one of the estrogenic compounds sharing structural features with 17β-estradiol (potent estrogenic compound), is a simple isoflavonoid occurring naturally in glycosylated forms.[96,97] It is widely used as a functional and nutraceutical food due to its various therapeutic potentials like antihelminthic and antioxidant effects. Genistein can bind to estrogen receptors with up to 7−8 times higher binding affinity.[98]

In recent years, high dietary intake of genistein has been linked to both memory and cognitive improvement in humans. These neuroprotective benefits are also demonstrated in an *in vivo* model associated with HD-like symptoms. Accordingly, systemic genistein administration (20 mg/kg, ip) increased retention latencies of 3-NP treated rats, further confirming the improvement in memory retention.[97] Furthermore, genistein protected neurons against oxidative stress-induced apoptosis by regulating the expression of Bax/Bcl-2. It is worth noting that genistein is effective in attenuating age-related memory and cognitive deficits via antiapoptotic and antioxidant activities.

Lutein. Lutein is a xanthophyll naturally found in plants such as marigold and green leafy vegetables and egg products.[99] When compared to other carotenoids, lutein warrants attention as a powerful antioxidant, a fact contributed by its unique chemical structure that possesses not only conjugated double bonds but also two hydroxyl groups on both ends, which strengthen its antioxidant activity.[100] Lutein (50 and 100 mg/kg, po) has been reported to have the ability to terminate the free radical reactions and protect neurons from 3-NP triggered oxidative stress in a rat model of HD-like symptoms in a dose-dependent manner.[100] The protective effects conferred restore the activity of the antioxidant defense enzymes and mitochondrial complex followed by the preservation of normal ATP function. Consequently, 3-NP-induced mitochondrial dysfunction is ameliorated. In addition, lutein normalizes the activity of AChE, thus improving memory function and cognitive task performance in 3-NP treated rats.

Lycopene. Another naturally occurring carotenoid, lycopene, is a red pigment present predominantly in tomatoes and tomato products. Lycopene is a potent antioxidant that can scavenge free radicals. Based on the findings of Sandhir et al.,[17] the neuroprotective effects of lycopene against the HD *in vivo* model were ascribed to (1) neutralization of free radicals, (2) restoration of antioxidant status in the brain and (3) the modulation of lipid peroxidation in 3-NP treated rats. Lycopene (10 mg/kg, po) also normalizes ETC function, thereby alleviating mitochondrial dysfunction and causing a more controlled release of mitochondrial cytochrome *c* into the cytosol, which may activate apoptosis. Finally, it is suggested that the therapeutic effects of lycopene are also contributed by the suppression of activation of MAPKs and NF-κB signaling pathways.

Nicotine. Nicotine, a natural alkaloid that originates from *Nicotiana tabacum* Linn (commonly known as tobacco), is composed of a pyrrolidine and pyridine ring. Moreover, nicotine crosses the blood−brain barrier and has some

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neuroprotective effects as seen in 3-NP-induced HD *in vivo* models. Tariq et al.[101] suggested that pretreatment of medium (0.5 mg/kg, ip) and high (1.0 mg/kg, ip) doses of nicotine significantly attenuated the depletion of striatal DA, followed by motor activity improvement in a dose-dependent manner. The pharmacological effects of nicotine likely to be mediated by the (1) involvement of neuronal nicotine acetylcholine receptor (nAChR) agonists[102] and (2) stimulation of BDNF, nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), as well as basic fibroblast growth factor (FGF-2).[103]

Túnez et al.[104] revealed another neuroprotective mechanism of low dose nicotine (1.5 mg/kg, ip) in which it exhibited antioxidant effects by inhibiting oxidative damage induced by 3-NP, suggesting that nicotine sequesters ferrous ion (Fe^{2+}) inhibiting the Fenton reaction. Moreover, nicotine enhances succinate dehydrogenase (SDH) activity by acting as a superoxide anion scavenger via inhibiting complex I of mitochondrial electron transport[105] and producing a monoaminergic neurotransmitter liberation on nACh receptors in cortical neurons. These protective actions synergistically restore energy metabolism in the rat's brain.

Praeruptorin C. *Peucedanum praeruptorum* Dunn is an herbaceous plant from Apiaceae family. Historically, the dried root of this plant, Peucedani Radix, is widely utilized in Chinese traditional medicine for the treatment of cough, airway infections, and hypertension.[106] Subsequently, it has been identified that praeruptorin C (PRA-C) is the main active constituent in dried root extract.[107] Research on PRA-C suggest that it exerts neuroprotective potency in attenuating the 3-NP-induced motor deficits, excitotoxicity, and depression in a HD *in vivo* model.[107,108] In a separate study, Wang et al.[107] reported that the administration of PRA-C (1.5 and 3.0 mg/kg, po) effectively alleviated abnormal glutamate release while decreasing calcium influx. Additionally, PRA-C efficiently improves a series of behavioral and neurochemical alterations and histological damage in the striatum by preventing the loss of cellular viability from striatal neurons. Keeping this fact in view, the researchers conjectured the possible beneficial effects of PRA-C to be due to the upregulated expression of DARPP-32 (dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32000), Htt, and BDNF in the amygdala, which are mechanistically related to the regulation of emotion and motor function.[109]

Puerarin. Radix puerariae (kudzu root), also known as Ge Gen in China, is the dried root of a medicinal plant, *Pueraria lobate* Ohwi. It is one of the earliest edible herbs in Oriental medicine.[110] Puerarin (PUR) is a type of isoflavonoid [1.88−2.55% (w/w)] isolated from radix puerariae. PUR has been traditionally used for centuries in the treatment of cardiovascular diseases, hypertension, and diabetes mellitus.[111] Much evidence has confirmed the fact that PUR can penetrate the BBB immediately following intravenous administration thus conferring good neuroprotective potency.[111,112] Owing to this fact, relevant studies involving PUR administration (200 mg/kg, ip) have been conducted in a 3-NP intoxicated HD *in vivo* model.[112,113]

Accumulated results confirmed the neuroprotective effect of PUR on the brain tissue against 3-NP-induced neurotoxicity, in terms of reduced degree of hypothermia and brain injury with enhanced levels of antioxidative enzymes.[113] The discovery is in agreement with previous reports in which PUR has been confirmed to protect hippocampal neurons from neurotoxicity by (1) enhancement of CAT activity and GPx level, (2) reduction of LPO level, and (3) decreased formation of ROS. Taken together, this suggests that PUR provides neuroprotection by virtue of its antioxidant ability and neuromodulative effects. On the other hand, Mahdy et al.[112] reported that pretreatment with PUR prevented the increase in pro-apoptotic biomarkers by down-regulating the ratio of Bcl-2-associated X protein (Bax)/B-cell lymphoma 2 (Bcl-2) and attenuating caspase-3 activation thus conserving the anti-apoptotic effects. In addition, treatment with PUR can (1) suppress NF-κB activation, (2) inhibit iNOS expression, and (3) normalize ATP levels, ultimately restoring the energy for metabolism. Therefore, overall, the antiapoptotic and anti-inflammatory properties of PUR may protect the brain tissues from oxidative stress-induced inflammation and apoptosis.

Quercetin. Quercetin is a natural dietary flavonoid present abundantly in most edible fruits and vegetables including red onions, black or green tea, grapes, broccoli, blueberries, and cranberries.[114] Quercetin possesses great pharmacological effects in preventing and treating cancer, cardiovascular disease and neurodegenerative disorders.[115] Interestingly, quercetin can cross the BBB and exert its neuroprotective effect. Chakraborty et al.[116] reported that quercetin (25 and 50 mg/kg, ip) can reduce 5-hydroxytryptamine (5-HT) levels markedly with a subsequent increase in striatal serotonin metabolism, possibly due to the inhibitory effect of monoamine oxidase A (MAO-A). Additionally, quercetin preserves mitochondrial function in 3-NP administered rats by a virtue of its antioxidant effects. Apart from this, the anti-inflammatory activity of quercetin has also been indicated by the decreased microglial activation and increased infiltration of astrocytes in the brain lesion core.

Resveratrol. Resveratrol, 3,4′,5-trihydroxystilbene, is a phytoalexin naturally found in various plants such as peanuts, berries, and grapes.[117] It is a nutraceutical that has useful anticancer, anticardiovascular disease, and antioxidant properties.[118] Kumar et al.[118] demonstrated that resveratrol (5 and 10 mg/kg, po) dose-dependently offers good protection against 3-NP-induced oxidative stress, improving both cognitive and motor performance of 3-NP treated rats. Due to the decreased oxidative stress, SDH activity was restored. Interestingly, resveratrol has been shown to decrease mitochondrial complex III activity in the respiratory chain of the rat's brain, which helps to directly maintain the stabilizing effect of the mitochondrial membrane, in turn, preserving mitochondrial function in the brain of 3-NP treated rats.

S-Allylcysteine. *S*-Allylcysteine is a compound that is an odorless, stable, and water-soluble organosulfur abundantly found in garlic.[119] It is derived from cysteine, an amino acid in which an allyl group is attached to the sulfur atom.[120] The antioxidant effect of *S*-allylcysteine has been demonstrated in a 3-NP-induced toxicity *in vivo* model.[121] *S*-Allylcysteine (300 mg/kg, ip) restores SOD activity and prevents lipid peroxidation by conferring primary protection of cells from 3-NP triggered oxidative stress. For the latter, the compound was found to prevent 3-NP-induced mitochondrial dysfunction and energy failure due to reduced ATP levels, thus suggesting that *S*-allylcysteine completely preserves cell survival and neuronal function from 3-NP-induced neuro- and excitotoxicities.

Sesamol. *Sesamum indicum* Linn (Pedaliaceae), also known as black sesame, is a herbaceous plant native to India and Africa. It is also cultivated in Asia mostly for its edible oil and

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seed. Traditionally, owing to its high degree of resistance to rancidity and oxidation, it is recognized as the "queen of oil seeds".[122] Sesamol is one of the phenolic compounds that is mainly extracted from *Sesamum indicum*. To date, it has been commonly used as a popular dietary supplement worldwide due to its antioxidant value, which is effective in preventing hyperlipidemia, hypertension, and atherosclerosis and demonstrates antiaging and anticancer properties.[38]

Motivated by the significant reported properties of sesamol, Kumar et al.[123,124] have conducted investigations to explore the neuroprotective potency of sesamol against HD in the 3-NP-induced rat model. Pretreatment with sesamol (5, 10, and 20 mg/kg, po) conferred significant neuroprotection against 3-NP-induced motor and cognitive dysfunction and cellular alteration in different regions of the rat brain. Restoration of mitochondrial enzymes and nonenzymatic and enzymatic antioxidants has contributed to the amelioration of oxidative damage in the hippocampus, cortex, and striatum of the brain. The researchers hypothesized that the effect of sesamol in alleviating neuroinflammation is contributed by its antioxidant properties.

Solanesol. In fact, tobacco extracts and isolated compounds have good therapeutic potential against neurodegenerative disease owing to their anti-inflammatory, antioxidant, anesthetic, antispasmodic, sedative, and anticonvulsant properties.[125] In this direction, great interest has been directed toward several polyphenols present in tobacco for therapeutic purposes, including solanesol (SNL), a precursor of coenzyme Q10, which was shown to possess neuroprotective effects in the HD *in vivo* model based on 3-NP intoxication.[126] Interestingly, SNL (5, 10, and 15 mg/kg, po) dose-dependently increased ATP levels to a large extent thereby enhancing mitochondria function. By neutralizing free radical formation and increasing the enzymatic activity of coenzyme Q10, it restored the antioxidant activity and also attenuated the inflammatory damage in different areas in the rat's brain. Additionally, SNL also restored the cholinergic function and brain histopathological changes. Moreover, the findings of Mehan et al. support the neuroprotective potential of SNL in ameliorating 3-NP-induced memory and cognitive loss and motor incoordination.[126]

Spermidine. Spermidine, one of the natural and ubiquitous polyamines, is an aliphatic polycation having a low molecular weight and nucleophilic centers. Meat, milk products, and green leafy vegetables are its major sources. Mounting evidence has revealed that spermidine has good antiaging effects, delaying the pathophysiology of neurodegenerative disorders.[127] Jamwal and Kumar[128] investigated the protective effects of spermidine in the 3-NP-induced *in vivo* model and found that pretreatment with spermidine (5 and 10 mg/kg, po) successfully ameliorated 3-NP triggered motor incoordination in a dose-dependent manner by (1) preventing the alteration of striatal neurotransmitters and (2) reducing the lipid peroxidation and oxidative stress followed by oxidative protein damage in rats. These protective effects could be well-correlated with the antioxidative potential of spermidine in scavenging free radicals in the rat brain. Owing to the anti-inflammatory properties, spermidine is capable of deactivating the MAPK and NF-κB signaling pathways.[127,128] Consequently, this phenomenon directly decreases the neuroinflammatory marker levels and striatal neuron degeneration in 3-NP treated rats, as well as prolonging the rat's lifespan.

Tetramethylpyrazine. *Ligusticum wallichii* Franchat (Chuan Xiong) is a traditional Chinese herb that belongs to the Umbelliferaceae family. It has been widely used in China in the treatment of cardiovascular and neurovascular diseases over the decades.[129] The active compound of Chuan Xiong, tetramethylpyrazine (TMP), has been reported to confer some neurotrophic and neurobiological effects while ameliorating HD-like symptoms in 3-NP-induced rats.[130] Therefore, attention is warranted for further investigation on TMP as a potential antioxidant against 3-NP neurotoxicity by inhibiting the elevation of lipid peroxidation (LPO) and scavenging the ROS.

In another study by Danduga et al.,[130] TMP (40 and 80 mg/kg, ip) dose-dependently attenuated 3-NP excitotoxicity by increasing the γ-aminobutyric acid (GABA) levels as well as reducing glutamate levels. Moreover, it ameliorated memory impairment in rats by virtue of its antidepressant effect,[131] attributed to the regulation of BDNF and the phosphorylation of CREB in the cAMP/PKA signaling pathway.[132] Interestingly, the neuroprotective potency of TMP is attributed to its efficacy in restoring cholinergic functions, restoring striatal DA, and improving mitochondrial biogenesis in the brain, all of which contribute to the enhanced cognitive and motor performance.[130]

L-Theanine. L-Theanine (γ-glutamylethylamide), which accounts for approximately 1.5% of the dry weight of tea leaves, is one of the major non-protein amino acids and is synthesized from glutamic acid and ethylamine in green tea leaves.[133] It is structurally similar to glutamate and GABA. L-Theanine can cross the BBB via a larger neutral amino acid transport system. It diminished the mitochondrial dysfunction in rats systemically administered 3-NP by preventing the reduction of LPO level and SDH activities, as well as by restoring the levels of enzymatic antioxidants, as confirmed by Thangarajan et al.[134] On the other hand, subchronic treatment with L-theanine (100 and 200 mg/kg, po) dose-dependently enhanced GABA synthesis, which triggers relaxation. Collectively, L-theanine mitigates 3-NP-induced oxidative stress by inhibiting NF-κB activation, which is accompanied by upregulated expression of BDNF.[135] Finally, it can be concluded that L-theanine offers its neuronal protection against 3-NP triggered neurotoxicity in rats via anti-inflammatory and antioxidant activities.

In Vitro and In Vivo Studies. Berberine. Berberine is a protoberberine alkaloid that can be derived from the bark and roots of *Berberis* sp. or *Coptis chinenses*. For over six decades, berberine has been widely used in Chinese medicine to treat diarrhea due to bacterial infection. To date, it has been indicated in the treatment of cardiovascular disease, hypercholesterolemia, inflammation, and diabetes. Its benefits include (1) a high tolerance oral dose [50% lethal dose (LD_{50}) > 5 g/kg] and (2) the ability to cross BBB. Due to these reasons, there is high interest to investigate its neuroprotective potential in both *in vitro* and *in vivo* models.[32] The findings indicated that berberine (40 mg/kg, po) can improve the motor function of transgenic mice in a HD *in vivo* model. It also confers neuroprotection by activating Nrf2 and glucagon-like protein (GLP-1) and inhibiting MAO-B and AChE. Interestingly, berberine (50 μM) can also enhance the autophagy process in parallel with increased degradation of mHtt in a HD *in vitro* model.

Gintonin. Gintonin is a novel lysophosphatidic acid (LPA)−ginseng protein complex naturally derived from

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ginseng.[136] It contains an exogenous G-protein-coupled lysophosphatidic acid receptor (LPAR) ligand capable of repressing inflammation. Studies have reported that oral administration of gintonin exhibited antidementia and antimetastatic effects[137] and enhanced memory, learning, and physical stamina.[138,139] However, the anti-inflammatory potential of gintonin remains unclear. Motivated by this reason, Jang et al.[139] investigated the anti-inflammation activity of gintonin against striatal toxicity in both *in vitro* and *in vivo* HD models. The findings from the HD *in vivo* model reveal that preadministration, coadministration, and onset administration of gintonin (100 mg/kg, po) reduce striatal cell death and attenuate neurological impairment by mitigating 3-NP-induced mitochondrial dysfunction, expression of pro-inflammatory cytokines, iNOS and cycloxygenase-2 (COX-2), and microglial activation. It was suggested that gintonin exerts its protective mechanism via the (1) inhibition of MAPKs, (2) NF-κB signaling pathways, (3) activation of LPAs, and (4) Nrf-2 pathways.[139] Additionally, the beneficial effects of gintonin (0.1, 1.0, and 10.0 μM) in a concentration-dependent manner were also reported in the adeno-associated virus (AAV) vector-infected STH*dh* cell HD *in vitro* model, reducing the neurological impairment via the mechanism of reduced formation and expression of mHtt aggregates.

Naringin. Naringin, 4,5,7-trihydroxy-flavonone-7-rhamnoglucoside, is an important major dietary flavanone glycoside found in citrus and grapefruits including *Citrus unshiu*, *Citrus sinensis*, *Citrus paradise*, *Poncirus* sp., and *Artemisia selengensis* due to its purported antioxidant, anti-inflammatory, antiatherogenic, and anticarcinogenic effects.[18] The neuroprotective potential of naringin against HD-mediated neurotoxicity has been shown in various studies.[18,140−142] The discoveries from both *in vitro* and *in vivo* reports support the hypothesis that naringin is the most potent Nrf2 inducer and protects neuronal cells against 3-NP-induced neurotoxicity by activating Nrf2.[18] Collectively, naringin (80 mg/kg, po) increases the nuclear accumulation of Nrf2, which directly induces Nrf2-regulated ARE gene expression. Subsequently, the activation of Nrf2 exerts a series of protective effects in multiple pathways including induction of the expression of phase II antioxidant genes, glutathione S-transferase P1 (GSTP1), γ-glutamylcysteine synthetase (GCS) mRNA, NQO1, and HO1.

On the other hand, naringin (10 μM) decreases the expression of 3-NP-induced matrix metalloproteinase-2 (MMP2), matrix metalloproteinase-9 (MMP9), NF-κB, and glial fibrillary acidic protein (GFAP), which indicates its potent neuroprotective effects in preventing striatal toxicity and oxidative damage in 3-NP treated rat brain as well as PC-12 cells.[141] In addition, naringin elicits its antioxidant effects by (1) potentiating the endogenous antioxidant defense capacity through an increase of SOD and GPx levels and CAT activity in cells and (2) preserving the mitochondrial respiratory chain integrity via a decrease in mitochondrial complex enzyme activities. Apart from the antioxidant properties, naringin possess antiapoptotic effects that can protect cells from neuronal apoptosis by decreasing the expression of Bcl-2-associated agonist of cell death (Bad) and Bax concomitant with a decrease in caspase-3 and cytochrome *c*.[140] Taken together, these protective activities contribute to cellular protection against 3-NP-induced hazardous effects.

Protopanaxatriol. *Panax ginseng* C.A. Mayer, ginseng from the Araliaceae family, is an ancient herb widely used in traditional Chinese medicine. Ginsenosides are important components responsible for the neuroprotective actions of ginseng, as also mentioned before. There are more than 30 different kinds of ginsenosides that can be isolated from *Panax ginseng* and are classified based on their chemical structures into (1) protopanaxatriols (Rg1, Rg2, and Re) and 2) protopanaxadiols (Rb1, Rb2, Rc, Rd, and Rg3).

Protopanaxadiol Rb1 can pass through the BBB. In an *in vitro* HD assay, Rb1, Rc, and Rg5 conferred some protection to cultured MSNs against glutamate-induced neurotoxicity.[66] The findings revealed that Rb1, Rc, and Rg5 at low concentrations (0.01 and 0.01 μM) successfully attenuated glutamate-induced apoptosis that accompanied the inhibition of neuronal Ca^{2+} signaling. Therefore, it is plausible that the neuroprotective effects of protopanaxadiols on the glutamate-induced HD *in vitro* model occurs due to the inhibition of the Ca^{2+} signaling pathway.

On the other hand, Gao et al.[143] conducted an *in vivo* study investigating protopanaxatriol (PPT) (10 mg/kg, po), which was found to confer some neuroprotection against 3-NP-induced behavioral, biochemical, and histological changes in Sprague-Dawley rats. PPT elicits its antioxidant effects via a series of mechanistic actions, including (1) free radical scavenging, (2) reduction of overproduced ROS, (3) inhibition of neuronal oxidative stress-induced reactions, and (4) elevation of activated endogenous antioxidant enzymes. Through these mechanisms, PPT directly restores the activity of the mitochondrial complex enzymes, subsequently protecting the mitochondrial function. By acting as a potent antioxidant agent, PPT upregulates the entry of nuclear factor erythroid 2-related factor 2 (Nrf2) into the nucleus along with the expression of heme oxygenase 1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO-1) which ultimately protects the neurons from 3-NP-induced neurotoxicity or striatum damage.

Sulforaphane. Sulforaphane, 1-isothiocyanato-4-(methylsulfonyl)-butane, is a dietary isothiocyanate correlated with antioxidant, antiapoptosis, antigenotoxicity, anticancer, and antimicroglial activation effects. It is also known as a chemopreventive agent most abundantly found in various cruciferous vegetables including cauliflower, brussels sprouts and broccoli. A mechanism of action of sulforaphane has been proposed by Liu et al.[144] based on an *in vitro* study. The study found that sulforaphane (2, 4, and 8 μM) concentration-dependently activates Atg pathways concomitant with autophagy activity, therefore synergistically promoting the removal and degradation of unwanted substrates in bulk.[144] Since sulforaphane can cross the BBB, it has dual effects of (1) maintaining protein homeostasis and (2) suppressing free radicals to protect the neuronal cells against the toxicity of 3-NP.

The compound is also reported to mitigate 3-NP-induced striatal neurotoxicity in a HD *in vivo* model.[145] In the study, pretreatment with sulforaphane (5 mg/kg, ip) effectively ameliorated the neurological impairment along with striatal lesion volume triggered by 3-NP intoxication by the activation of Kelch-like ECH-associated protein 1 (Keap1)−Nrf2−antioxidant response element (ARE) [Keap1−Nrf2−ARE] pathway and the inhibition of MAPK and NF-κB pathways. Together with these signaling pathways, sulforaphane suppresses 3-NP-induced oxidative stress, SDH activity, microglial activation, apoptosis, the elevation of iNOS, expression of COX-2, and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). Additionally, the activated Nrf2−ARE

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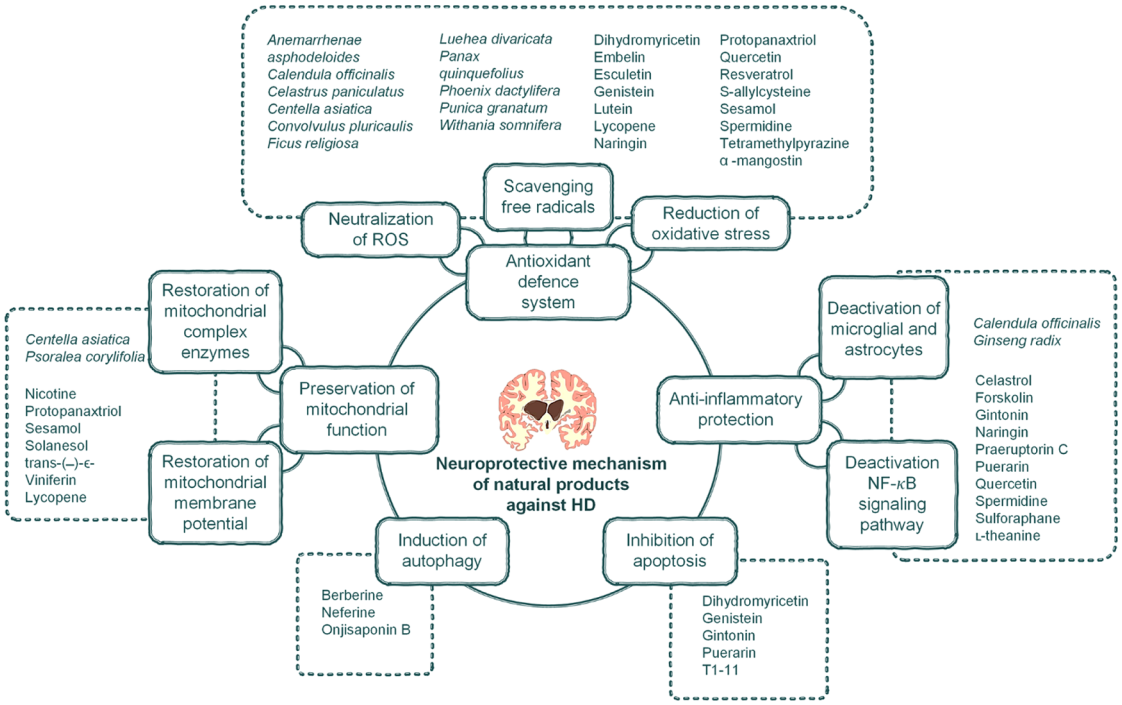

Figure 6. Possible neuroprotective mechanism of natural products against HD in experimental models.

pathway sequentially induces phase II metabolizing antioxidant enzymes to exert antioxidant effects. The degradation of mHtt and ubiquitinated proteins via the UPS pathway is also enhanced by the peripheral injection of sulforaphane.[145]

T1-11. *Gastrodia elata* Blume is from the Orchidaceae family. It has a long history recorded in Chinese Pharmacopeia for 1500 years. The dried tuber of *Gastrodia elata* is used to treat dizziness, headaches, limb numbness, spasms, and convulsive illness such as tetanus and epilepsy.[146,147] To date, the literature reports two compounds, especially vanillin and gastrodin, that possess anticonvulsive, antiepileptic, and sedative properties. Two other active compounds, namely, bis(4-hydroxylbenzyl)sulfide and *N*-(4-hydroxybenzyl)adenine riboside (T1-11), have also been shown to protect PC-12 cells from apoptosis, confirming the central protective effects of *Gastrodia elata*.

Huang et al.[147] demonstrated the neuroprotective effects of T1-11 (4.66 μM) by an *in vitro* assay, showing decreased mHtt aggregation and increased proteasome activity in neuronal PC-12 cells indicating its potential in HD. Similar neuropharmacological effects of *Gastrodia elata* extract were also reported in a transgenic HD *in vivo* model. T1-11 (0.05 mg/kg, ip) acted via the adenosinergic system in synapses by activating the adenosine A_{2A} receptor ($A_{2A}R$) and is therefore recognized as a potent adenosine analog that performs dual functions of (1) activating $A_{2A}R$ and (2) blocking the adenosine transporter. Both activities synergistically inhibit endogenous adenosine uptake to exert a neuroprotective mechanism. Additionally, activation of $A_{2A}R$ also effectively facilitates the function of certain neurotrophic factors, including BDNF, GDNF, and FGF.[147]

POTENTIAL NEUROPROTECTIVE MECHANISMS

To gain insight into the underlying mechanism of natural products and compounds on HD-like symptoms (Figure 6), there were numerous *in vitro* and *in vivo* studies conducted. They generally involved the antioxidant defense system, scavenging free radicals, neutralization of ROS, reduction of oxidative stress, preservation of mitochondrial function, anti-inflammatory protection, inhibition of apoptosis, and induction of autophagy. ROS, including hydroxyl radicals ($OH^{\bullet}$), superoxide (O_2^-), and hydrogen peroxide (H_2O_2) are continuously generated during cellular aerobic respiration.[148,149] ROS (1) plays a decisive role in modulating the biological processes of nerve cells in counteracting endogenous antioxidant defense status in the CNS and $OH^{\bullet}$ involving multiple biological reactions and (2) is responsible for the oxidative damage to proteins, DNA, and lipids, while O_2^- take part in the production of H_2O_2.[149]

Physiologically, the levels of ROS generated are in equilibrium with the cellular antioxidant status. Nevertheless, when the level overwhelms the endogenous antioxidant capacity, a state of oxidative stress, cellular oxidative damage in the brain might ensue.[148] This condition is one of the convergent mechanisms closely correlated with the pathogenesis of HD and other neurodegenerative diseases since the CNS region is rich in polyunsaturated fatty acids where a high consumption of oxygen leads to a higher vulnerability to oxidative stress.

Interestingly, natural products (*Punica granatum* and tetramethylpyrazine) have been demonstrated to possess antioxidative properties in 3-NP treated brain and in PC-12 cells.[43,130] Mounting evidence has indicated that these natural products are capable of sequestering free radicals, reversing the decreased level of enzymatic and nonenzymatic antioxidants, and in turn attenuating the elevated level of cellular oxidative stress. In reacting to the oxidative stress, a compensatory mechanism of endogenous antioxidative defense system is also induced by activation of Nrf2. The activated Nrf2–ARE signaling pathway sequentially induces the metabolizing antioxidant enzymes in exerting antioxidant effects by upregulating the enzymatic antioxidants (SOD, CAT, and

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GPx) and nonenzymatic antioxidants (GSH), to neutralize the excess ROS in the brain. Many studies revealed that SOD and its isoforms, Zn-, Fe-, Cu-, and Mn-dependent SOD (Zn-SOD, Fe-SOD, Cu-SOD and Mn-SOD), preferably detoxify O_2^- by transforming it to H_2O_2. To detoxify H_2O_2, CAT is responsible for converting it to H_2O. In addition, GSH, GPx, γ-glutamyl-cysteinyl-ligase (GCL), glutathione reductase (GSSR), and glutathione transferase (GST) also play a vital role in detoxifying H_2O_2 to H_2O.[150] Collectively, lipid peroxidation is reduced and these activities can directly protect the distinctive cells and neurons against 3-NP-induced oxidative damage.[47] Furthermore, AChE level is reduced owing to the antioxidant capacity of forskolin, lutein, and berberine, suggesting the anticholinergic ability of these natural products in offering neuroprotection.[32,100]

Mitochondria are the main engine for ATP production, which involves a series of oxidative phosphorylation reactions in the mitochondrial respiratory chain. High oxygen and glucose consumption in the brain make neurons more dependent on ATP generation within the mitochondria. Along the mitochondrial respiratory chain, mitochondrial complex enzymes I, II, IV, and V play a pivotal role in ATP synthesis. Interestingly, some natural products like *Withania somnifera*, epigallocatechin gallate, and sesamol have been shown to upregulate the enzyme activity of NADH, SDH, cytochrome oxidase, and F_1F_0 synthase.[69,124,134] The restored mitochondrial complex enzyme activities are capable of normalizing ATP synthesis. Moreover, certain natural compounds such as genistein and naringin can mitigate glutamate-induced Ca^{2+} overloads on the NDMA receptor, thereby preserving ATP production.[48,66] Additionally, natural products like *Psoralea corylifolia* can restore mitochondrial membrane potential ($\Delta\Psi$) by normalizing Ca^{2+} influxes and ATP synthesis, thus preventing inner and outer membrane damage and mitochondrial dysfunction, as well as preserving the metabolic rate in mitochondria along with maintaining ETC integrity.[39]

It is noteworthy that a number of natural products like *Calendula officinalis* Linn and puerarin possess anti-inflammatory activities that are responsible for decreasing neuroinflammation.[45,112] The pathological mechanism of HD extensively involves the elevation of pro-inflammatory markers (TNF-α, IL-1β, and IL-6), which directly activate the NF-κB signaling pathway together with the activation of microglia and astrocytes. In response to the inflammatory conditions, certain natural products can impact the microglia by inhibiting the activation of glial cells and astrocytes and by blocking the NF-κB pathway, thereby attenuating the delivery of pro-inflammatory cytokines to the immune system in HD preclinical models.[80,145] Apart from inhibiting inflammatory pathways, the reduced expression of these cytokines, especially TNF-α can inhibit HD-induced apoptotic pathways as a result of elevated levels of TNF-α and its binding on TNFr1-type receptors, leading to the activation of apoptotic cascades.

A plethora of studies suggested that natural products such as puerarin elicit antiapoptotic effects by (1) upregulating the expression of Bcl2 and (2) decreasing the expression of Bad and Bax, both concomitant with the blocking of cytochrome *c* release via the deactivation of caspase-3.[18,112] BDNF, NGF, GDNF, and FGF-2 are a group of neurotrophins that are beneficial for neuronal survival by preventing apoptosis. In this context, natural products have a role in stimulating the expression of these neurotrophins, DARPP-32, and Htt, thus mechanistically inhibiting the neuronal apoptosis.[103,146] Many natural products have an antiapoptotic ability, which may also contribute to attenuation of decreased levels of catecholamines (dopamine, norepinephrine, and serotonin) in striatum nuclei.[151]

Autophagy is a cellular self-degradation and removal process for dysfunctional cytoplasmic components. It was reported that natural compounds (onjisaponin B, neferine, and sulforaphane) have been shown to reduce 3-NP-induced neurotoxicity via the induction of mTOR−AMPK dependent autophagy activities.[74,77,145] The findings support a working model of natural products to ameliorate the neuronal damage by activating the autophagy activities dependently on the gene regulation of *Atg7* via the mTOR−AMPK signaling pathway. Consequently, the aggregation of mHtt was shown to be reduced by autophagy in cultured PC-12 cells, and the cognitive function of rats in the HD *in vivo* model was enhanced. Accumulating literature provides proof of neuroprotective potential from various natural products to attenuate HD-like symptoms under *in vitro* and *in vivo* models via single or combined neuropharmacological mechanisms.

CHALLENGES AND OPPORTUNITIES IN THE FIELD OF NATURAL PRODUCTS AGAINST HD

Medicinal plants and their isolated compounds have potential to be a valuable resource for drug discovery against HD. In this review, although several preclinical experiments have shown therapeutic promise, human HD clinical trials remain a challenge and are severely lacking. In translating the promising preclinical research to clinical applications, there are several difficulties and constraints such as poor water solubility of the natural products, their physicochemical properties, prompt metabolism, and low bioavailability. In addition, the existence of the BBB restricts their passage to the brain and some specific sites of action.

Nevertheless, since some reported isolated compounds in this review (lutein, lycopene, naringin, quercetin, resveratrol, *S*-allylcysteine, and sulforaphane) are widely found in fruits and vegetables commonly consumed, phase I clinical trials can be intiated, since these food products are deemed as safe for human consumption. Additionally, most of the medicinal plants reviewed have been traditionally used for many years in Ayurvedic medicine, traditional Chinese medicine, and other traditional medical systems. Another recent trend is the enrichment of foods and beverages, where fortification techniques using natural products may be a potential prevention strategy for HD. Although natural products should be tested in preclinical studies before evaluating their effect on humans in randomized clinical trials, which can help assess safety, tolerance, and efficient therapeutic doses for disease treatment, this poses a challenge.

Low bioavailability is one of the key drawbacks associated with natural products, which restricts their delivery to the target for pharmacological action. The use of nanotechnology and nanocarrier-based methods in the delivery of natural products may help solve these problems and enhance therapeutic responses while improving their efficacies. In fact, the integration of nanoparticles into brain-focused drug delivery systems (DDSs) is a promising strategy to increase the bioavailability and transport of natural products through the BBB.[152−156] DDSs can protect natural products from biological degradation when the molecules are transported into the brain.[157] Thus, low doses of natural products can steadily

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be released into the brain for enhanced efficacies. Nose to brain drug delivery (NBDD) is another useful method for improving the absorption, bioavailability, and therapeutic effects of natural products for treating brain disorders.[158] Growing evidence has indicated that nasal administration is a possible direct route to prevent BBB from blocking transport of drugs into the brain and has high bioavailability for amelioration of brain diseases.[158] In addition to the above-mentioned DDSs, new pharmaceutical technologies such as liposomal nanoencapsulation, polymeric micelles, cyclodextrins, nanosuspensions, and nanoemulsions are needed to increase the bioavailability of natural products, resistance to metabolic processes, and passage through the BBB.[159−164]

In another aspect, drug metabolism and pharmacokinetics (DMPK) research is essential for understanding the efficacy and safety of natural products against HD. The reported compounds may be structurally modified to improve the DMPK properties, and such modifications may help to increase the activity strength and selectivity, improving solubility and partition coefficient, increasing metabolic and chemical stability, modulating pharmacokinetic parameters, and removing or alleviating toxicity and adverse reactions. To accomplish these multidimensional operations, sophisticated syntheses and skillful preparation of complicated molecules are essential. Finally, the mutual cooperation and interaction between organic and medicinal chemists are crucial in modifying natural products for commercial use through drug research and development. For the drug discovery process of HD, the isolated compounds listed in this review must be filtered using the Lipinski and Veber rules on the basis of drug-like properties.[165,166] Further, it is important to filter the listed compounds by removing substructures with Pan Assay Interference Compounds (PAINS) to avoid false positives.[167,168] All these findings together can be used to discover a potential lead compound against HD based on common natural products.

METHODS

For this review, relevant studies were collected from several scientific databases including PubMed, ScienceDirect, Scopus, and Google Scholar. The categories of keywords used for the search included

(a) "Natural product" OR "Medicinal plants" OR "Plant extract" AND "Huntington's disease".

(b) "Natural compounds" OR "Phytochemicals" AND "Huntington's disease".

After screening literature from 2005 to the present, a total of 14 plant species and 30 isolated natural compounds that have been investigated against HD based on either *in vitro* or *in vivo* models were included in the present review.

CONCLUSION AND FUTURE PERSPECTIVES

This review presents a comprehensive account of the neuroprotective efficacy of various natural products against HD experimental models. Fourteen medicinal plants and 30 isolated compounds described herein have been shown to be effective against HD. Most of the medicinal plants listed in this review are traditional medicinal plants used for the treatment of brain disorders. The majority of the isolated compounds mentioned in this review are primarily from common plant species, are present or incorporated into traditional medicinal plants, or are found in food sources including in fruits, herbs, and spices. Since HD is a multifactorial disease, only natural products identified with distinct therapeutic mechanisms were included in this review.

Interestingly, promising *in vitro* and *in vivo* evidence has accumulated that *Anemarrhenae asphodeloides* (0.5 and 1.0 μg/mL) and *Centella asiatica* (5 mg/kg, po) are potentially effective at low concentrations or doses compared to other medicinal plants to protect against 3-NP-induced HD. Among the isolated compounds mentioned, proropanaxatriol (0.01 and 0.1 μM) and nicotine (0.25, 0.50, and 1.0 mg/kg, ip) are potentially effective when used in low concentrations or doses against 3-NP-induced HD. Apart from these, all the other natural products reported in this review also can ameliorate altered behavioral, biochemical, and histopathological parameters, indicating their potential effectiveness based on *in vitro* and *in vivo* HD models. For most of the reported natural products, the possible modes of protection have also been extensively studied. Conclusively, accumulated evidence has implicated the model of natural products in HD preclinical models working mainly through the antioxidant defense system, preservation of mitochondrial function, anti-inflammatory protection, inhibition of apoptosis, and induction of autophagy. Nevertheless, several perspectives on the application of natural products for the prevention and treatment of neurogenerative potential are suggested. In addition to conducting further research to understand how natural products exert their therapeutic effects on HD, it is also necessary to undertake additional experimentation to target only certain natural products acting on the brain. Although natural products have vast therapeutic potential against HD, most are confirmed only in early phases of study. Randomized controlled trials are required to further strengthen the claims.

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Author Contributions

P.T.L. and M.S. designed and conceived the ideas, collected the literature, interpreted the data, analyzed the data, and drafted and revised the manuscript. S.H.G., S.R.B., and M.F.S. analyzed the data and revised the manuscript. P.T.L., M.S., S.R.B., and M.F.S. created the figures. All authors read and approved the final manuscript.

Notes

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■ ABBREVIATIONS

3-NP, 3-nitropropionic acid; 5-HT, 5-hydroxytryptamine; AAV, adeno-associated virus; $A_{2A}R$, adenosine A_{2A} receptor; AC, adenyl cyclase; AChE, acetylcholinesterase; AMPK, AMP-activated protein kinase; ARE, antioxidant response element; ASO, antisense oligonucleotides; Atg, autophagy related; ATP, adenosine triphosphate; Bad, B-cell lymphoma 2 (Bcl-2)-associated agonist of cell death; Bax, Bcl-2-associated X protein; BBB, blood−brain barrier; BDNF, brain-derived neurotrophic factor; CAG, cytosine-adenine-guanine; cAMP, cyclic adenosine monophosphate; CAT, catalase; CBP, cAMP-CREB-binding protein; CGN, cerebellar granule neuron; CNS, central nervous system; COX-2, cycloxygenase-2; CREB, cAMP response element binding protein; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPR-Cas9, CRISPR-associated system 9; CS, citrate synthase; DA, dopamine; DARPP-32, dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32000; DDS, drug delivery systems; DMPK, drug metabolism and pharmacokinetics; Drp1, dynamin-1-like protein; ECGC, epigallocatechin gallate; ELT, escape latency; ERK, extracellular signal-regulated kinase; ETC, electron transport chain; FDA, Food and Drug Administration; FGF, fibroblast growth factor; FST, forced swimming test; GABA, γ-aminobuytric acid; GCL, γ-glutamyl-cysteinyl-ligase; GCS, γ-glutamylcysteine synthetase; GDNF, glial cell line-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; GLP-1, glucagon-like protein; GFP, green fluorescent protein; GFPu, GFP-UPS reporter; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, glutathione disulfide; GST, glutathione S-transferase; GSTP1, glutathione S-transferase P1; HD, Huntington's disease; HO1, heme oxygenase-1; HSF1, heat shock factor 1; HSP, heat shock protein; HSP70, heat shock protein 70; Htt, huntingtin; ICR, Institute of Cancer Research; ig, intragastric gavage; ip, intraperitoneal injection; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; KA, kynurenic acid; Keap1, Kelch-like ECH-associated protein 1; LC3-I, cytosolic form of LC3; LC3-II, LC3-phosphatidylethanolamine conjugate; LDH, lactate dehydrogenase; LKB1, liver kinase B1; LPA, lysophosphatidic acid; LPO, lipid peroxidation; MAO-A, monoamine oxidase A; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MDH, malate dehydrogenase; MEF, mouse embryonic fibroblast; mGluR5, metabotropic glutamate receptor subtype 5; mHtt, mutant huntingtin; mMP, mitochondrial membrane potential; MMP2, matrix metalloproteinase-2; MMP9, matrix metalloproteinase-9; MMP, matrix metalloproteinase; MSN, medium spiny neurons; mTOR, mammalian target of rapamycin; MWM, Morris water maze test; N2a, neuro-2A; nAChR, nicotine acetylcholine receptor; NAD, nicotinamide adenine dinucleotide; NADH, NAD^+ hydrogen (H); NE, norepinephrine; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NGF, nerve growth factor; NIT, nitrite; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NPSH, non-protein thiols; NQO1, NAD(P)H:quinone oxidoreductase 1; NR2B, NMDA receptor subtype 2B; Nrf2, nuclear factor erythroid 2-related factor 2; NBDD, nose to brain drug delivery; OFT, open field test; po, per os, oral administration; p53, tumor protein 53; p62, nucleoporin 62; PAINS, pan assay interference compounds; PC-12, pheochromocytoma-12; PKA, protein kinase; polyQ, polyglutamine; PPI, prepulse inhibition; PSD-95, postsynaptic density 95; PSH, protein thiols; PUR, puerarin; RNAi, RNA interference; RNS, reactive nitrogen species; ROS, reactive oxygen species; sc, subcutaneous injection; SD, Sprague−Dawley; SDH, succinate dehydrogenase; SIRT3, sirtuin-3; SNL, solanesol; SOD, superoxide dismutase; Sp1, specific protein-1; TATA, thymidine-adenine-thymidine-adenine; TBARS, thiobarbituric acid reactive substances; TBP, TATA-binding protein; TH, tyrosine hydroxylase; TIMP, tissue inhibitor of metalloproteinases; TL, transfer latency; TMP, tetramethylpyrazine; TNF-α, tumor necrosis factor α; TSH, total thiols; TST, tail suspension test; TSTQ, time spent in target quadrant; UPS, ubiquitin proteasome system; VCM, vacuous chewing movement; WT, wild-type; ZFP, zinc finger motif protein

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