Neuroprotective Potency of Mangiferin Against 3-Nitropropionic Acid Induced Huntington's Disease-Like Symptoms in Rats

Summary

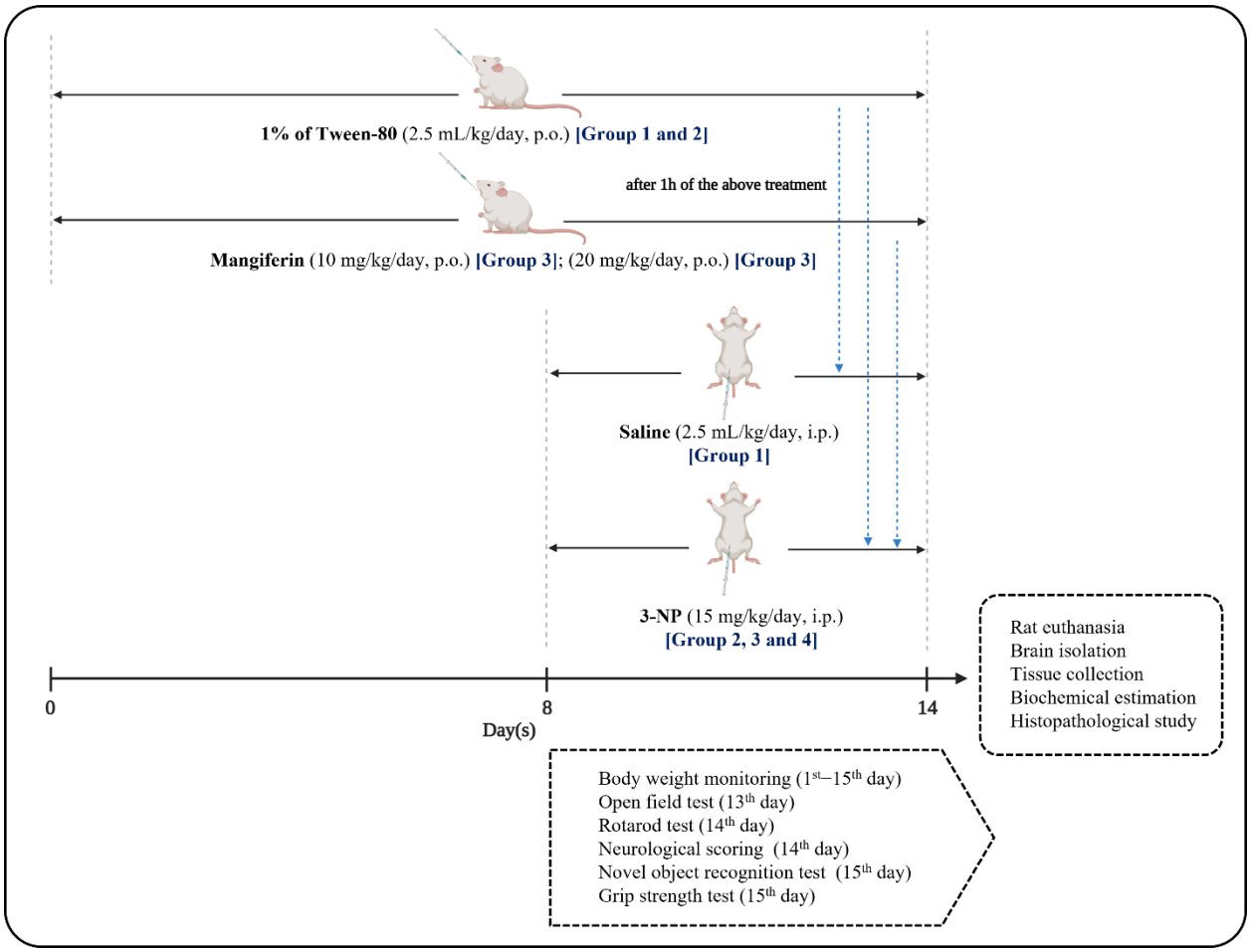
Huntington's disease (HD), a neurodegenerative disease, normally starts in the prime of adult life, followed by a gradual occurrence of psychiatric disturbances, cognitive and motor dysfunction. The daily performances and life quality of HD patients have been severely interfered by these clinical signs and symptoms until the last stage of neuronal cell death. To the best of our knowledge, no treatment is available to completely mitigate the progression of HD. Mangiferin, a naturally occurring potent glucoxilxanthone, is mainly isolated from the *Mangifera indica* plant. Considerable studies have confirmed the medicinal benefits of mangiferin against memory and cognitive impairment in neurodegenerative experimental models such as Alzheimer's and Parkinson's diseases. Therefore, this study aims to evaluate the neuroprotective effect of mangiferin against 3-nitropropionic acid (3-NP) induced HD in rat models. Adult Wistar rats (n = 32) were randomly allocated equally into four groups of eight rats each: normal control (Group I), disease control (Group II) and two treatment groups (Group III and Group IV). Treatment with mangiferin (10 and 20 mg/kg, p.o.) was given for 14 days, whereas 3-NP (15 mg/kg, i.p.) was given for 7 days to induce HD-like symptoms in rats. Rats were assessed for cognitive functions and motor coordination using open field test (OFT), novel object recognition (NOR) test, neurological assessment, rotarod and grip strength tests. Biochemical parameters such as oxidative stress markers and pro-inflammatory markers in brain hippocampus, striatum and cortex regions were evaluated. Histopathological study on brain tissue was also conducted using hematoxylin and eosin (H&E) staining. 3-NP triggered anxiety, decreased recognition memory, reduced locomotor activity, lower neurological scoring, declined rotarod performance and grip strength were alleviated by mangiferin treatment. Further, a significant depletion in brain malondialdehyde (MDA) level, an increase in reduced glutathione (GSH) level, succinate dehydrogenase (SDH), superoxide dismutase (SOD) and catalase (CAT) activities, and a decrease in tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) levels were observed in mangiferin treated groups. Mangiferin also mitigated 3-NP induced histopathological alteration in the brain hippocampus, striatum and cortex sections. It could be inferred that mangiferin protects the brain against oxidative damage and neuroinflammation, notably via antioxidant and anti-inflammatory activities. Mangiferin, which has a good safety profile, may be an alternate treatment option for treating HD and other neurodegenerative disorders. The results of the current research of mangiferin will open up new avenues for the development of safe and effective therapeutic agents in diminishing HD.

Background

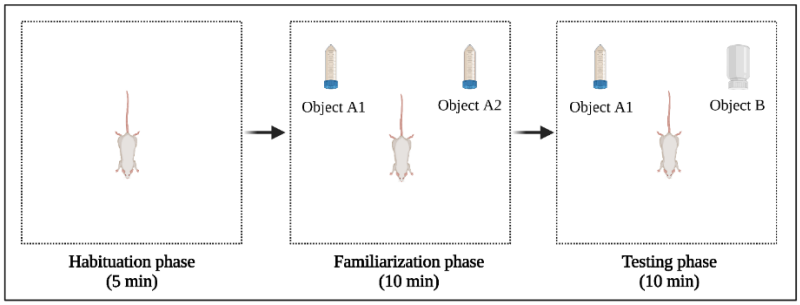
Mangiferin (molecular formula: $C_{19}H_{18}O_{11}$; molecular weight: 422 g/mol; structural name: 1,3,6,7-tetrahydroxyxanthone C2-β-D-glucoside) (Figure 2) is a naturally occurring pharmacologically glucoxilxanthone which being extensively investigated for its biological and therapeutic potentials. Accordingly, Walia et al. (2020) reported that mangiferin is widely present in 96 species, 28 genera and 19 families of angiospermic plants. Mangiferin has also been found in certain monocots and ferns such as *Acystopteris sp.*, *Asplenium adiantum-nigrum, Cystopteris sp., Davallia subsolida*, *Gymnocarpium sp.*, *Trichomanes reniforme* and *Woodsia sp.*, as well as in young leaves of *Coffea pseudozanguebariae* (Matkowski et al., 2013; Sekar, 2015; Jyotshna et al., 2016). However, *Mangifera indica* (Family: Anacardiaceae), commonly known as Mango which is abundantly available in Malaysia, is the primitive and chief source of mangiferin. Despite of the exact mechanism of mangiferin absorbed through blood−brain barrier (BBB) is unknown, an intense effort has been

devoted to evaluating the therapeutic potential of mangiferin against neurological disorders (Feng et al., 2019; Walia et al., 2020; Liu et al., 2021). Accumulating studies implicate that mangiferin offers neuroprotection to the CNS against mitochondrial dysfunction, oxidative stress, cellular apoptosis and neuroinflammation. It also improves the memory loss and declined cognition of rats under memory impairment *in vivo* model (Lum et al., 2020). Building on the information from the literature, mangiferin appears to be a promising agent against HD. Furthermore, understanding the neurotherapeutic efficacy of mangiferin with its underlying mechanisms is of great significance. Owing to a paucity of research of mangiferin on HD, the present study was conducted to evaluate the neuroprotective efficacy of mangiferin against 3-NP induced HD in rats.

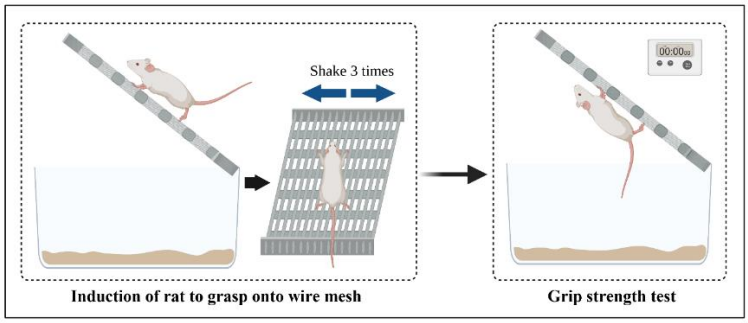
Experimental Design



Experimental design

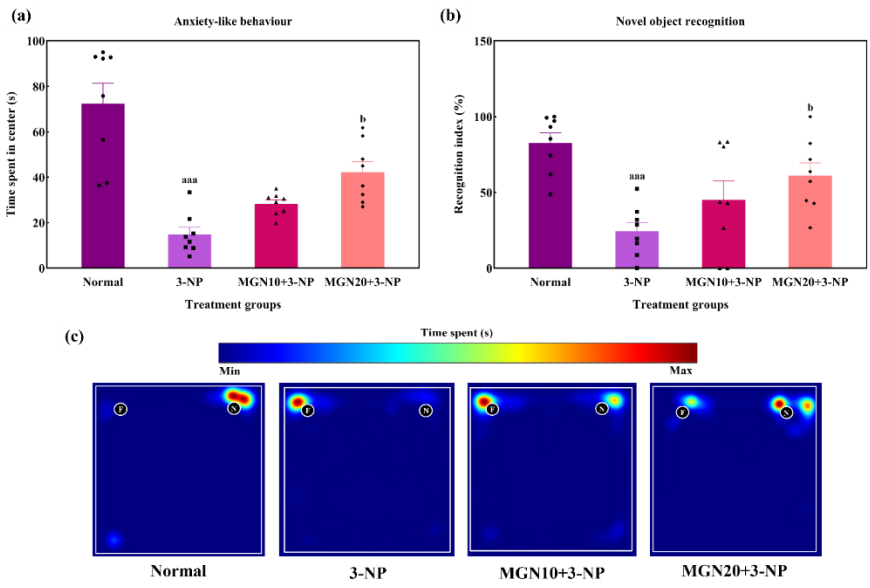


Novel object recognition experimental protocol

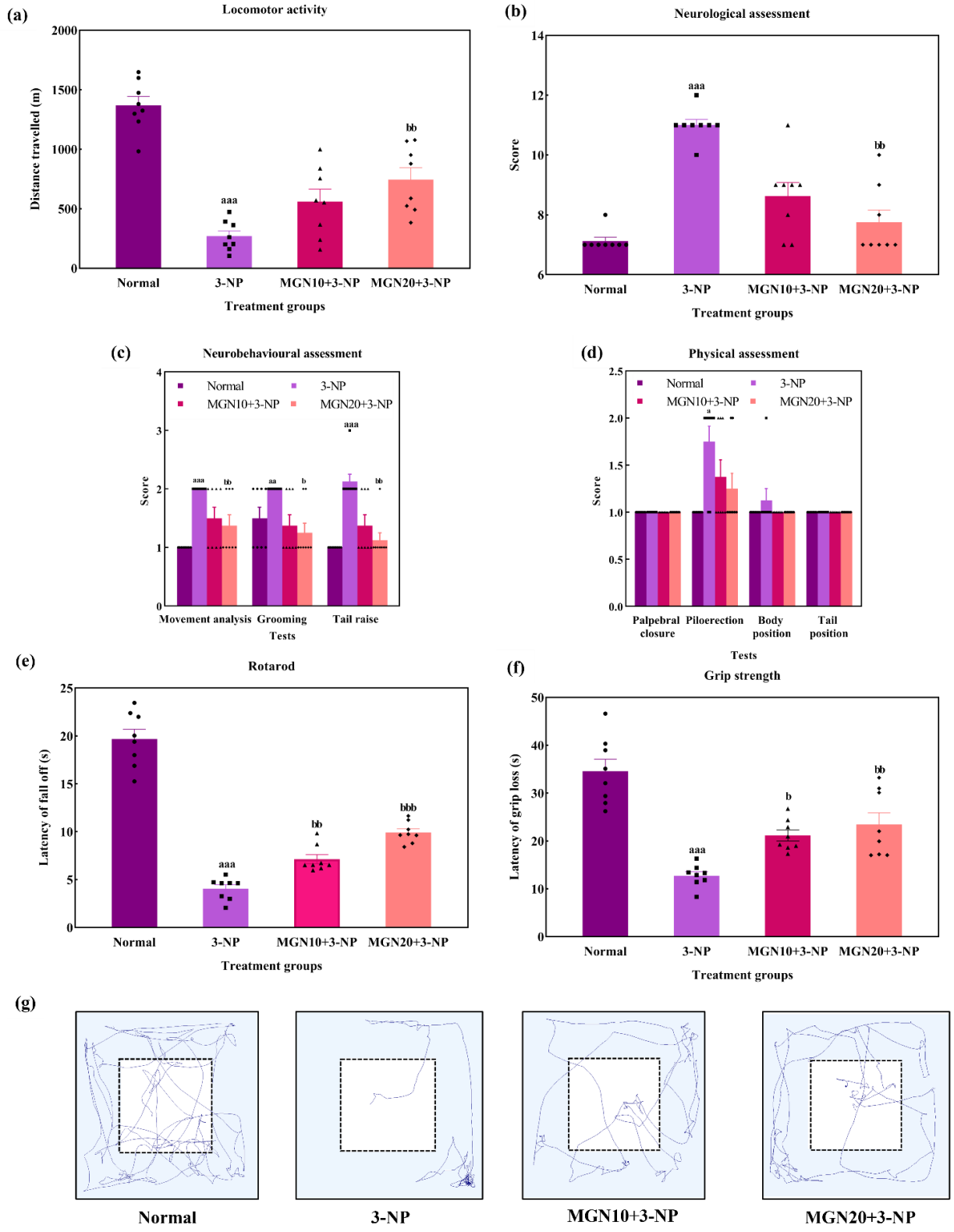


Schematic illustration of grip strength test

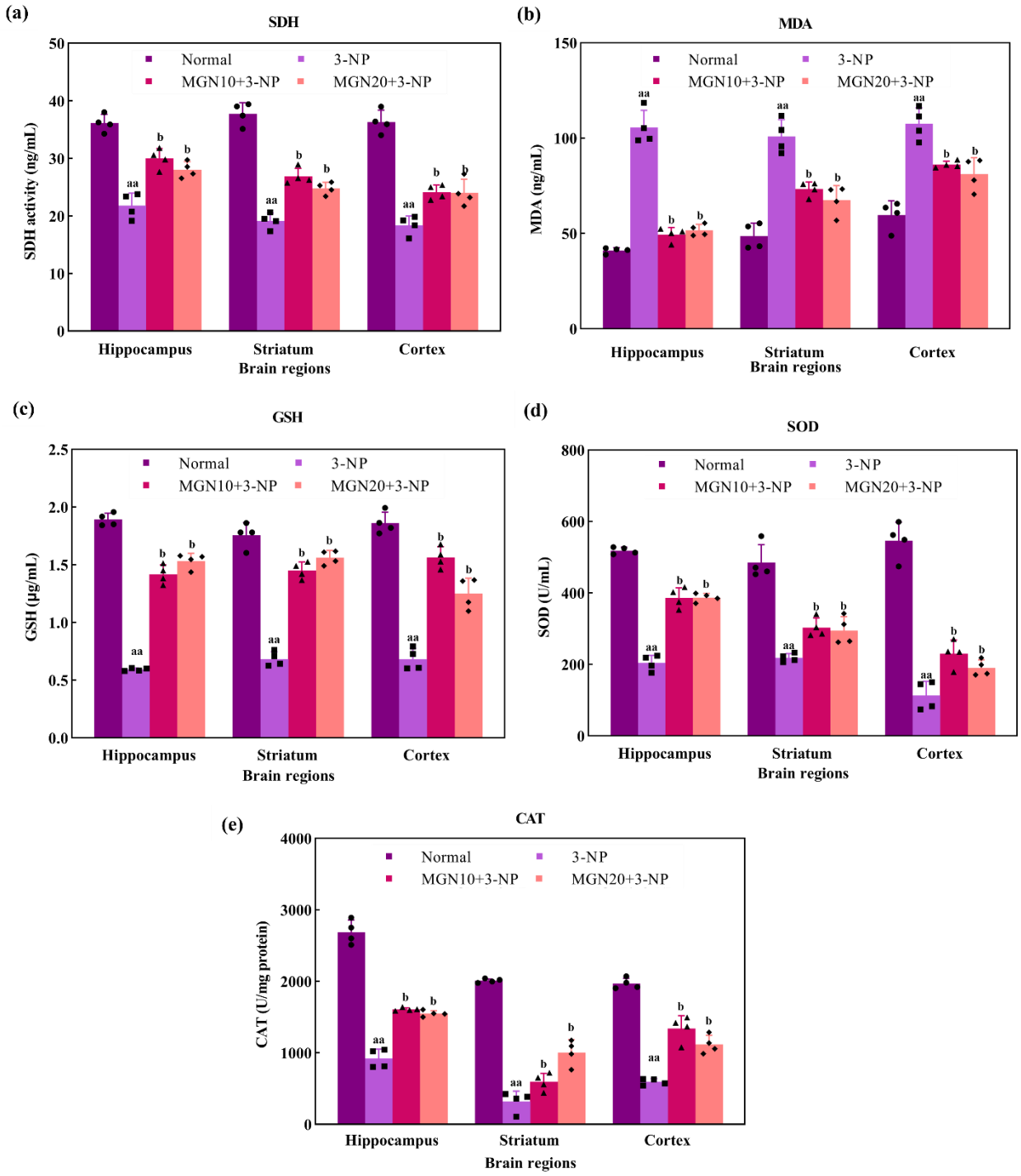
Results



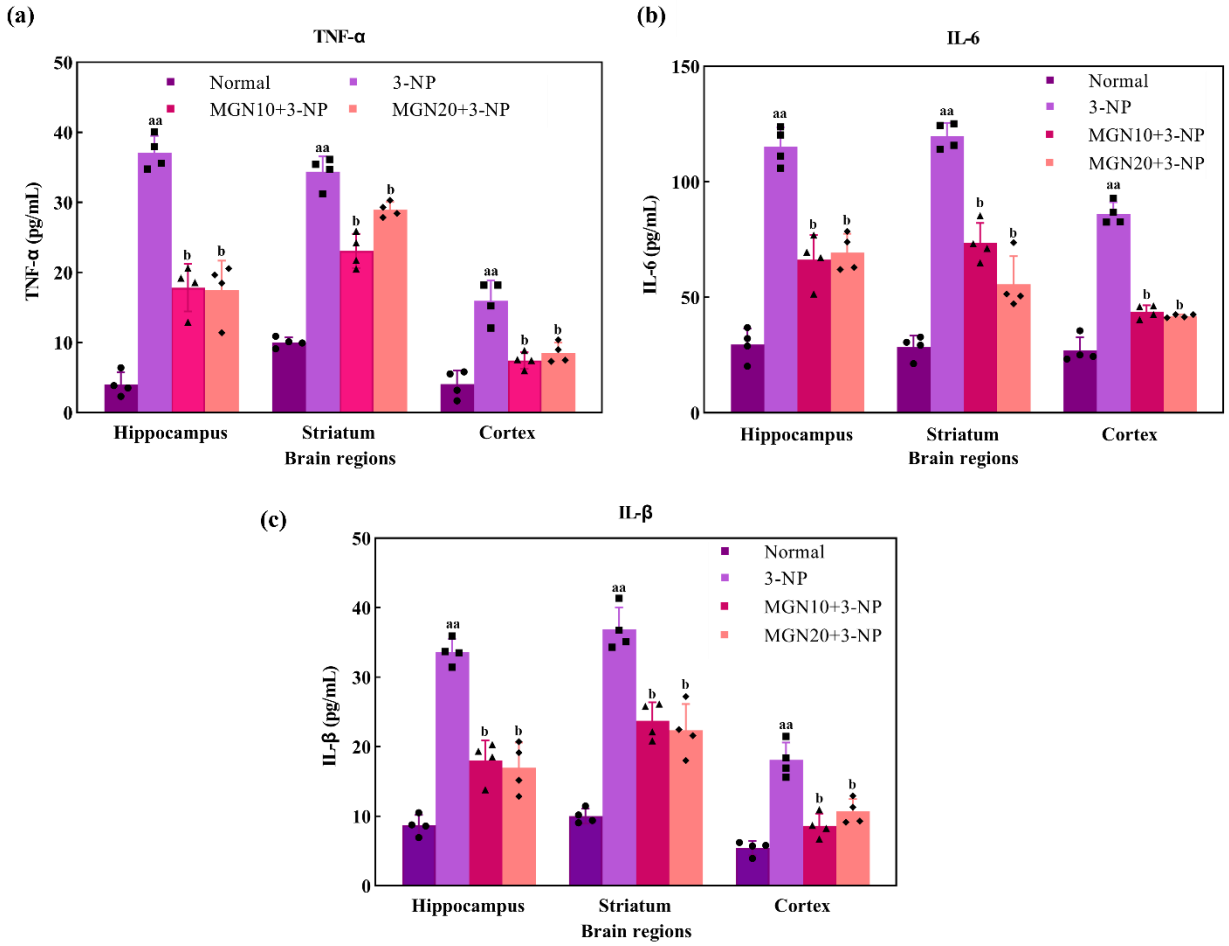
Effect of mangiferin on (a) anxiety-like behaviour and (b) NOR. (c) Representative heat map visualisation in NOR test, indicating time spent and location near the objects. Data represents mean ± SEM, n = 8 per group. ^{aaa} $p < 0.001$ compared to normal control group; ^{b} $p < 0.05$ compared to 3-NP alone treated group.



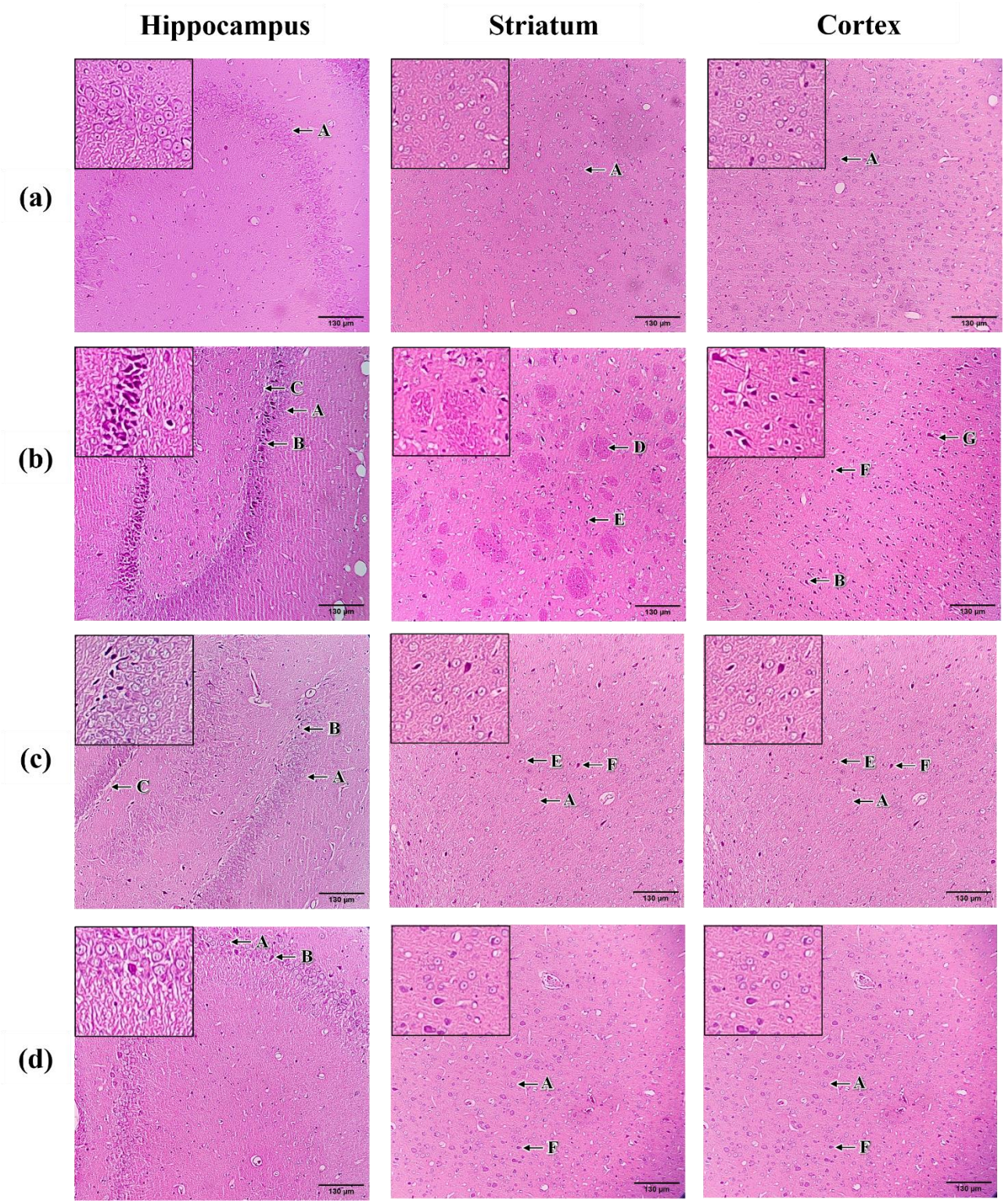
Effect of mangiferin on (a) locomotor activity, (b) total neurological scoring, (c) neurobehavioural and (d) physical assessments, (e) rotarod, and (f) Grip strength. (g) Representative track visualisation of rats in open field arena. Data represents mean ± SEM, n = 8 per group. ^{a} $p < 0.05$, ^{aa} $p < 0.01$, ^{aaa} $p < 0.001$ compared to normal control group; ^{b} $p < 0.05$, ^{bb} $p < 0.01$ and ^{bb} $p < 0.01$ compared to 3-NP alone treated group.



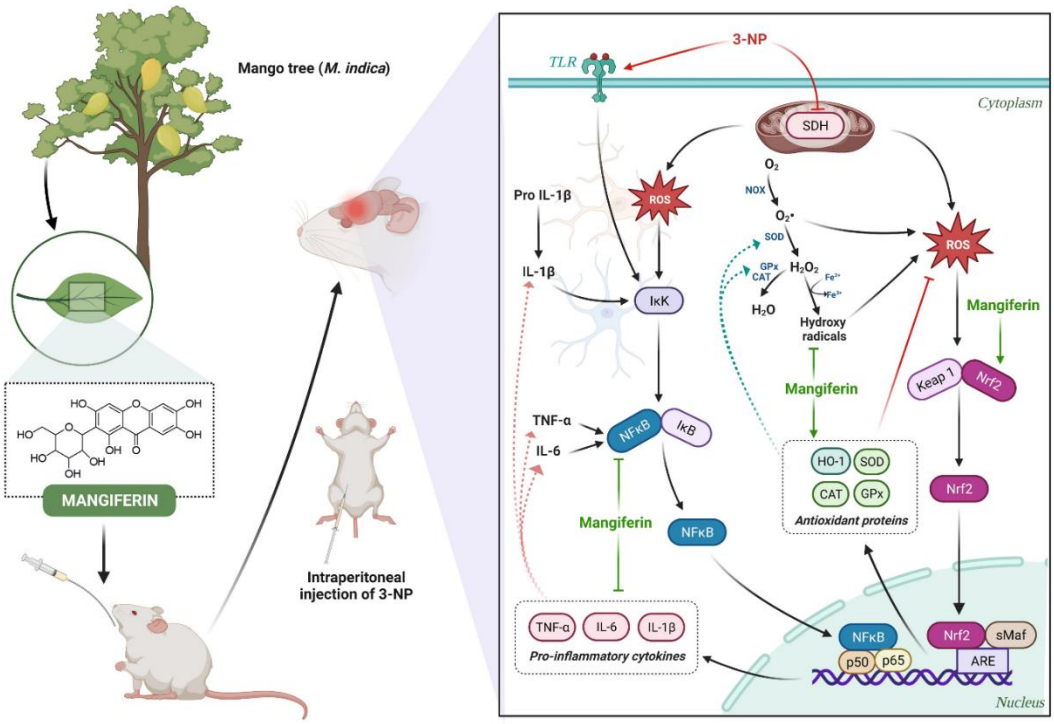
Effect of mangiferin on oxidative stress markers: (a) SDH activity, (b) MDA, (c) GSH level, (d) SOD and CAT activities. Data represents mean ± SEM, n = 4 per group. ^{aa} $p < 0.01$ compared to normal control group; ^{b} $p < 0.05$ compared to 3-NP alone treated group.



Effect of mangiferin on pro-inflammatory markers: (a) TNF-α, (b) IL-6 and (c) IL-1β level. Data represents mean ± SEM, n = 4 per group. ^{aa} $p < 0.01$ compared to normal control group; ^{b} $p < 0.05$ compared to 3-NP alone treated group.



Photomicrographs of H&E-stained brain sections of hippocampus, striatum, and cortex with a magnification of 100X for (a) normal control, (b) 3-NP, (c) mangiferin (10 mg/kg) + 3-NP, and (d) mangiferin (20 mg/kg) + 3-NP groups. A: Normal pyramidal cells, B: Necrotic pyramidal cells, C: Intercellular oedema; D: Excess degenerative plaque, E: Pyknotic neuronal cells; F: Neuronal degeneration, and G: Shrunken and necrotic neuronal cells with neurofilbrillary tangles.



Neuroprotective mechanism of mangiferin via antioxidant and anti-inflammatory pathways against 3-NP induced HD rat model.

Discussion

This study demonstrated that mangiferin (1) alleviated 3-NP induced body weight changes (increased body weight gain), behavioural deficits (improved anxiety-like behaviour, recognition memory, locomotor activity, neurological scoring, rotarod performance and grip strength), (2) restored 3-NP induced biochemical alteration (decreased MDA level, increased level of GSH, enhanced SDH, SOD and CAT activities), and (3) mitigated 3-NP induced histopathological changes (mild neuronal degeneration) in rats. 3-NP, an irreversible inhibitor of SDH, effectively produces HD-like symptoms such as striatal degeneration along with cognitive and motor abnormalities in rat models. Although the underlying mechanism of action of 3-NP is not clearly understood, but oxidative stress-driven mechanisms, including mitochondrial dysfunction, neuroinflammation, and excitotoxicity were proposed (Singh et al., 2015; Bhangale et al., 2016; Mehan et al., 2018). Therefore, 3-NP induced model was used to investigate the neuroprotective efficacy of mangiferin in HD *in vivo* model. In this regard, 3-NP sub-chronic administration at 15 mg/kg/day, i.p. for 7 days, substantially caused poor body weight gain in rats. The present finding is similar to previous literature as rats treated with 3-NP triggered a reduction in body weight (Dhadde et al., 2016; Karandikar and Thangarajan, 2017). These results could be attributed to 3-NP induced mitochondrial dysfunction following depressed cellular energy metabolism (Binawade and Jagtap, 2013) and sustainable metabolic alteration in the brain (Túnez et al., 2010), mimicking consistent features in the initial phase of HD patients. Accordingly, 3-NP irreversibly inhibits SDH activity in the Krebs cycle and electron transport chain (Malik et al., 2022), simultaneously interrupts the activities of mitochondrial complexes along with the loss of membrane potential and mitochondrial dysfunction, which ultimately causes adenosine triphosphate (ATP) depletion and impaired energy metabolism (Túnez et al., 2010).

Intriguingly, mangiferin treatment for 14 days markedly prevented poor weight gain in 3-NP treated rats, indicating the restoration of SDH activity and mitochondrial function by mangiferin owing

to its antioxidant effects. Recovered energy metabolism sequentially improved weight gain in rats. This finding is evidenced by the biochemical data showing that mangiferin administration reversed the declined SDH activity in 3-NP treated rats. An emerging study supports the investigation that mangiferin recovered isoproterenol-induced body weight changes towards the normal range in heart failure rats (Jiang et al., 2020). Besides, the report suggests that mangiferin exerted regulatory effects on body weight in streptozotocin-induced diabetic animal models (Wu et al., 2021).

In agreement with the previous findings (Danduga et al., 2018; Sharma et al., 2021; Salman et al., 2022), in this study, memory and cognitive impairment were encountered by 3-NP treated rats. The rats revealed higher anxiety and hampered recognition memory with decreased recognition index following 3-NP administration. It has been postulated that the memory and cognitive deficits caused by 3-NP are mainly ascribed to the neuronal loss in the hippocampal regions CA1 and CA3 (Danduga et al., 2018), which is critical for episodic and spatial memory (Dimsdale-Zucker et al., 2018). 3-NP intoxicated anxiety was due to dysfunction of striatal neurons (Sharma et al., 2021). Also, 3-NP was found to produce lesions specifically in the brain regions involving cognition, including the hippocampus, striatum and cortex (Dhadde et al., 2016). In this context, accumulating evidence suggests that 3-NP induced oxidative stress was considered a culprit for brain lesions and neuronal loss in HD (Túnez et al., 2010). The cognitive disturbances in HD are said to be subcortical dementia due to the nature of brain pathology involving striatal−subcortical pathways (Nopoulos, 2022). As observed clinically in HD patients, cognitive abilities decline progressively for years before the diagnosable motor onset of HD (Bates et al., 2015). In contrast to memory storage problems, HD patients are more likely to have slow recognition and trouble with memory retrieval (Bates et al., 2015; Ross and Margolis, 2022).

Along with cognitive deficits, 3-NP intoxicated rats exhibited motor abnormalities which were showed by reduced locomotor activity, shorter latency to fall from rotarod, and decreased grip strength. Partial limb reflex of rats was also observed in 3-NP alone treated group, as an indicator of neurological dysfunction (Davuljigari et al., 2021). These results are in tune with the previous studies (Thangarajan et al., 2014; Karandikar and Thangarajan, 2017; Moghaddam et al., 2021) as 3-NP caused deficiencies in locomotor and motor function by affecting the striatum, which is essential for body movement control (Kaur et al., 2016). The abnormalities were associated with marked neurodegeneration in striatum (Chakraborty et al., 2014) and cell gliosis (Suganya and Sumathi, 2017) induced by overwhelming oxidative/nitrosative stress in 3-NP action (Sharma et al., 2021).

Importantly, mangiferin treatment significantly mitigated 3-NP triggered memory and cognitive disabilities in rats, which is evidenced by reduced anxiety with increased time spent in center and restored spatial memory with higher recognition index to the novel object. As similar in previous literature, mangiferin treated mice (20 and 40 mg/kg, p.o.) shows reduced anxiety and depression from the results of open field test, light-dark box and elevated plus maze in the lipopolysaccharide (LPS) induced model (Jangra et al., 2014). Likewise, mangiferin was shown to exert significant protection on motor coordination in 3-NP treated rats, highlighted by improved locomotor activity, enhanced rotarod performance, increased grip strength and restored neurological behaviours. These findings are in accordance with the previous studies as growing evidence implicates the potential of mangiferin in alleviating anxiety and depressive-like behaviours, memory and learning deficits, and movement deficiencies by virtue of antioxidant and anti-inflammatory properties (Feng et al., 2014).

Oxidative stress is known as a chief determinant of neurotoxicity in HD (Bates et al., 2015). In this study, 3-NP intoxication significantly disrupted energy metabolism via inhibition of SDH activity. Elevated oxidative stress was also observed in 3-NP treated rats, as evident from the increased MDA level, diminished level of GSH and reduced activities of enzymatic antioxidants such as SOD and CAT in the hippocampus, striatum and cortex regions. These results are in agreement with previous studies (Mahdy et al., 2014; Danduga et al., 2018), as 3-NP has been reported to increase the generation of hydroxyl free radicals and initiate the oxidative cascade activation in the brain (Shetty et al., 2015). It

was also reported that oxidative stress mediated cell membrane damage and cell function loss lead to lipid peroxidation (Mehan et al., 2017). In regard to 3-NP induced neurotoxicity, oxidative metabolism induced by 3-NP triggers the sensitization of N-methyl-D-aspartic acid (NDMA) receptors and glutamate excitotoxicity, synergistically prompt an increase in intracellular calcium levels, resulting in further neuronal degeneration (Túnez et al., 2010). The alteration of dopamine and glutamate interactions is a great concern in HD. There is evidence that dopamine and glutamate neurotransmissions are affected in HD (André et al., 2010). In HD patients, it has been stated that abnormal extracellular dopamine levels with elevated glutamate levels lead to excitotoxicity, loss of striatal and cortical neurons, thereby develop to the later symptoms such as dystonia or akinesia (André et al., 2010).

The levels of pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1β were also markedly increased in the brain regions of hippocampus, striatum and cortex for 3-NP intoxicated rats. As documented in the previous findings, 3-NP treatment indicates an increase in microglial activation and mRNA expression of TNF-α, IL-6, IL-1β, inducible NOS (iNOS) and cyclooxygenase-2 (COX-2) in the striatum (Jamwal and Kumar, 2016; Jang et al., 2019). Recent literature has reported that high level of mHtt in monocytes and microglia as well as the accumulation of activated microglia and reactive astrocytes are observed in the brain of HD patients (Valadão et al., 2020), suggesting that microglia activation is one of the major sources of cytokine modulation in HD (Palpagama et al., 2019). It has been stated that mHtt aggregate positively activates microglia and induces the release of pro-inflammatory mediators such as TNF-α, IL-6, IL-1β and IL-8 via NF-κB signaling, with an increase of anti-inflammatory mediators such as IL-4 and IL-10 but at the later stages of HD (Tai et al., 2007; Björkqvist et al., 2008). Persistent microglia activation along with prolonged inflammatory cytokines production could lead to chronic inflammation in brain (Ellrichmann et al., 2013). Further, aggregation of mHtt in astrocytes results in reactive astrogliosis followed by glutamate excitotoxicity, contributing to HD neuronal death (Khakh et al., 2017).

In view of the abovementioned, the results from behavioural studies were further supported by the results from biochemical estimation. Oral administration of mangiferin (10 and 20 mg/kg, p.o.) in rats greatly protected the brain regions of the hippocampus, striatum and cortex against 3-NP induced oxidative stress. The SDH activity was found to be enhanced by mangiferin in 3-NP treated rats. As well, a significant decrease in MDA level along with an increased level of GSH and restored activities of SOD and CAT in the hippocampus, striatum and cortex were observed in mangiferin treated rats. The present findings are in line with the previous investigation of various natural products such as solanesol (Mehan et al., 2018), embelin (Dhadde et al., 2016), spermidine (Jamwal and Kumar, 2016), lutein (Binawade and Jagtap, 2013) and lycopene (Sandhir et al., 2010) on HD *in-vivo* models. Similarly, the study of (Kavitha et al., 2013) also reveals that mangiferin (10, 20 and 40 mg/kg, p.o.) ameliorates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced oxidative stress with reduced brain lipid peroxidation and GSH level in Parkinson's disease (PD) mice model. The prophylactic effect of mangiferin in reversing these alterations in biochemical parameters is due to the antioxidant activity in sequestering free radicals and enhancing brain cellular antioxidant defence (Sandhir et al., 2010; Mahdy et al., 2014). In Alzhemier's disease (AD) *in vitro* model, it has been stated that mangiferin effectively suppress β-amyloid (Aβ) induced neurotoxicity on brain cell via scavenging of ROS (Sethiya and Mishra, 2014). Ample reports implicate that mangiferin possesses free radicals scavenging activity in neuroprotection was mainly ascribed to its nature of C-glucosy linkage along with the presence of polyhydroxy components (Walia et al., 2020; Liu et al., 2021).

Besides, mangiferin treatment was shown to attenuate the elevation in TNF-α, IL-6 and IL-1β levels in the brain hippocampus, striatum and cortex, indicating the anti-inflammatory effect of mangiferin against 3-NP induced neuroinflammation. Consistent with these results, other studies also support the working model of mangiferin on neurodegenerative diseases, in which it could ameliorate the neuroinflammatory responses by declining the expression of IL-6 and IL-1β (Feng et al., 2019). In

the study of Luo et al. (2017), mangiferin treatment (40 mg/kg, p.o.) mitigated corticosterone triggered an increased level of TNF-α and IL-6 in the hippocampus. Similarly, mangiferin administration (20 and 40 mg/kg, p.o.) depleted $AlCl_3$ induced elevated release of TNF-α and IL-1β (Kasbe et al., 2015). It has been postulated that mangiferin reduces the secretion of pro-inflammatory cytokines by inhibiting the activation of astrocytes and microglia via the NF-κB signaling pathway (Infante-Garcia et al., 2017). In this context, it has been reported that mangiferin suppresses microglial activation by downregulating the synthesis of COX-2 and prostaglandin E2 (PGE-2) in both LPS induced *in vitro* (Bhatia et al., 2008) and *in vivo* (Jangra et al., 2014) models, indicating the vital role of mangiferin to protect the neurons from the neuroinflammatory response.

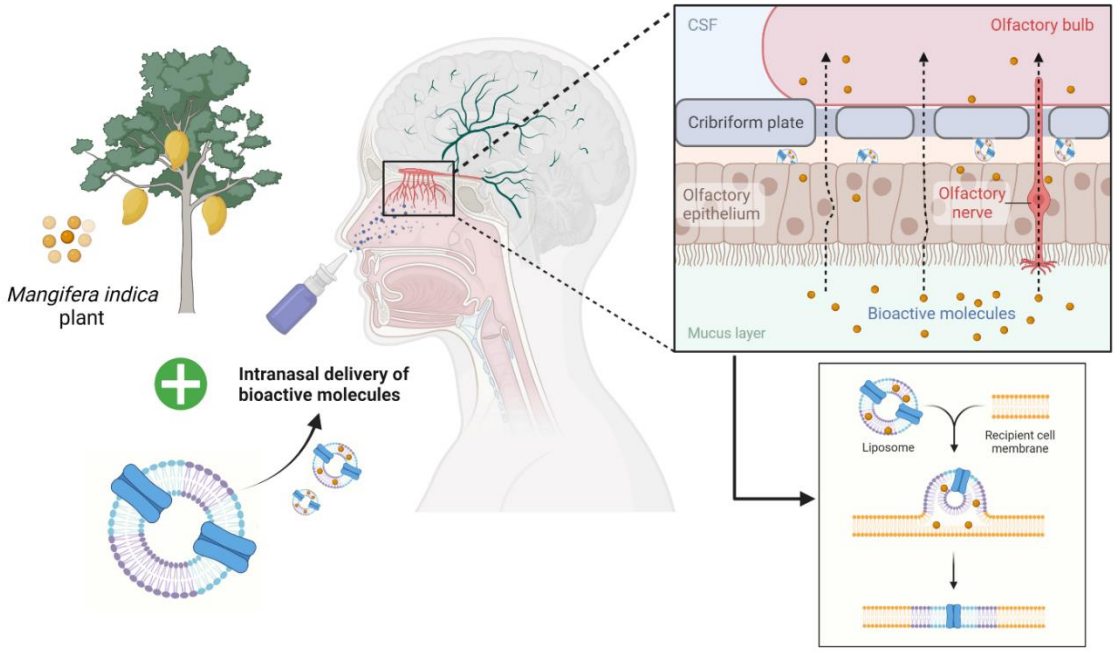
Apart from this, the neuroprotective potential of mangiferin against 3-NP induced HD rat model was further evaluated by histopathological examination of the brain regions of the hippocampus, striatum and cortex. In concordance with previous reports (Mehan et al., 2017; Mehan et al., 2018), in this study, histopathological changes with marked neurodegeneration were produced by 3-NP in the brain hippocampus, striatum and cortex of rats. These results further affirm that 3-NP induced cognitive and motor impairments were due to neuronal dysfunction. In contrast, the promising protective effect of mangiferin was confirmed with the mild degenerative changes in the brain tissue of the hippocampus, striatum and cortex in mangiferin treated groups. Supporting the histopathological results, 3-NP induced behavioural deficits and biochemical changes were greatly improved by mangiferin in this study.

Mounting pieces of evidence have indicated that mangiferin exerts its antioxidant mechanism in neuroprotection by 1) sequestering free radicals (Kasi et al., 2010), 2) reversing the decreased level of cellular antioxidants (Amazzal et al., 2007), 3) attenuating the elevated level of cellular oxidative stress by neutralizing excess ROS (Feng et al., 2017). The schematic diagram representing the antioxidant and anti-inflammatory mechanism of mangiferin are depicted in Figure 10. In reacting to oxidative stress, a compensatory mechanism of the endogenous antioxidative defence system is induced by mangiferin via the activation of the Nrf2 signaling pathway. Nrf2 is a redox-sensitive transcription factor that essentially regulates the ARE-driven gene expression of antioxidant proteins (Gil and Rego, 2008). The activated Nrf2-ARE signaling pathway sequentially induces the metabolizing antioxidant enzymes in exerting antioxidant effects by upregulating the enzymatic antioxidants (SOD, CAT, and GPx) and nonenzymatic antioxidants (GSH), to neutralize the excess ROS in the brain (Li et al., 2011). Heme oxygenase 1 (HO-1), a rate-limiting enzyme with the most abundant AREs, is also regulated by Nrf2 to catalyse the oxidative degradation of heme to biliverdin, produce carbon monoxide and ferrous ion in scavenging ROS (Wang et al., 2017b). Taken together, lipid peroxidation is reduced and these activities can directly protect the distinctive cells and neurons against 3-NP-induced oxidative damage (Malik et al., 2017).

Accordingly, neuroinflammation also occurs in the pathological mechanism of HD. In response to the immune reactions in the central nervous system, astrocytes and microglia are activated and an increased level of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) is secreted to impair the neurons (Stephenson et al., 2018). NF-κB is a transcription factor that plays a vital role in the mediation of cell proliferation, immunity, inflammation and survival (Liu et al., 2021). The inhibitor of NF-κB (IκB), the upstream factor of NF-κB, is activated via the phosphorylation of IκB kinase (Alam et al., 2021). Extensive studies suggest that mangiferin could inhibit the degradation of IκB and hinder the activation of NF-κB, to regulate the transcription of several inflammatory cytokines-related genes upon inflammation (Feng et al., 2019; Liu et al., 2021). Along with this, activation of astrocytes and microglia is inhibited by blocking the NF-κB pathway, thereby further attenuating the delivery of pro-inflammatory cytokines to the immune system in HD (Fu et al., 2014).

In conclusion, the present study indicated the neurotherapeutic potential of mangiferin against 3-NP induced body weight, behavioural, biochemical and histopathological alteration in rats. This provides a new insight into the working model of mangiferin as a neuroprotective agent for 3-NP induced HD-

like symptoms by virtue of antioxidant and anti-inflammatory activities. Mangiferin may replace conventional medicines in the treatment and prevention of HD due to its excellent safety profile. However, human research findings are insufficient. Thus, further dedicated works on clinical efficacy are recommended to envisage to its short- or long-term medicinal uses for improvement in HD. In the future, mangiferin can also be incorporated into nanocarriers such as liposomes and given intranasally (Figure 11). The liposomes encapsulating the bioactive compound will be exocytosed in the olfactory bulb after being endocytosed by the olfactory sensory cells. Then, the olfactory neurons replicate this trans-synaptic process, transferring the biomolecules to different parts of the brain to effectively reduce the progression of the disease.



Future prospective of employing liposomes as an intranasal delivery of bioactive molecules across the olfactory bulb.

Declaration

I am herewith declaring that the above research work is original work and not been given any award in the past

Dr. Mahendran Sekar
PhD (IND). M.Pharm (IND). B.Pharm (IND)
Associate Professor
School of Pharmacy
Monash University Malaysia

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