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Review

Neuroprotective effects of chrysin: From chemistry to medicine

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ABSTRACT

The World Health Organization estimated that the proportion of older people (over 60 years) will increase from 11% to 22% during next 40 years throughout the world. With respect to this, the morbidity and mortality rates of age-related diseases will increase. Mental diseases are the most common and important health problems among elderly people. Therefore, much attention has been paid to the discovery of neuroprotective drugs with high efficacy and negligible adverse effects. A growing body of scientific evidence has shown that phytochemicals possess neuroprotective effects and also mitigate neurodegeneration under both *in vivo* and *in vitro* conditions. Polyphenolic compounds, especially flavonoids, are known as most common chemical class of phytochemicals which possess a multiple range of health promoting effects. Chrysin, belonging to the flavone class, is one of the most important bioactive constituents of different fruits, vegetables and even mushrooms. Chrysin possesses potent neuroprotective effects and suppress neuroinflammation. In addition, chrysin improves cognitive decline and possesses a potent anti-amyloidogenic and neurotrophic effects. Furthermore, beneficial effects of chrysin on both depression and epilepsy have been reported. The present paper aimed to critically review the available literature data regarding the neuroprotective effects of chrysin as well as its chemistry, sources and bioavailability.

Contents

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1. Introduction

Flavonoids are the largest group of plant secondary metabolites with immense biological activities desired for human health. The research on flavonoids has been going on with growing interest as they act through physiological mechanisms and a large number of signalling pathways involved in many diseases. It has been estimated that the dietary flavonoid intake varies between 50 and 800 mg per day or greater, if dietary supplements are administered (Pietta, 2000). Among them, chrysin is a hydroxylated flavone derivative mainly found in honey, propolis and many plant species e.g. *Pelargonium crispum* (P.J. Bergius) L Her., *Passiflora incarnata* L., *Oroxylum indicum* (L.) Vent., *Scutellaria immaculata* Nevski ex Juz., *Scutellaria ramosissima* M. Pop., *Desmos cochinchinensis* Lour., *Cytisus multiflorus* (L'Her. Ex Aiton) Sweet., *Centaurea omphalotricha* (Batt.) Willk., *Lactarius deliciosus* (L. ex Fr.) S.F. Gray., *Suillus bellinii* (Inzenga) Watling., *Passiflora caerulea* L. (Bajgai et al., 2011; Escuredo et al., 2012; Mamadalieva et al., 2011; Mouffok et al., 2012; Pasini et al., 2013; Pereira et al., 2012; Sobočanec et al., 2006; Sulaiman et al., 2011; Williams et al., 1997; Yan et al., 2011). Previous studies have evaluated the concentrations of chrysin in several honeys. The chrysin content is 0.10 mg/kg in honeydew honey, and 5.3 mg/kg in forest honeys (Hadjmohammadi et al., 2010). Another study showed that the chrysin content in propolis is as high as 28 g/L (Pichichero et al., 2010). Individual chrysin content in a variety of mushrooms from the island of Lesvos, Greece ranged between 0.17 mg/kg in L. deliciosus to 0.34 mg/kg in *S. bellinii* (Kalogeropoulos et al., 2013).

Chrysin has been shown to be a very active flavonoid exerting a vast number of pharmacological properties such as anti-inflammatory activity *via* blocking histamine release and pro-inflammatory cytokine expression (Bae et al., 2011), anti-asthmatic activity through suppression of inducible nitric oxide synthase (iNOS) and nuclear factor-κB (NF-κB) (Wadibhasme et al., 2011), anticancer activity by endorsing the cell death induced by tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and increasing TRAIL-induced degradation of caspases 3 and 8 (Li et al., 2011), inhibition of histone deacetylase (Sun et al., 2012) and DNA topoisomerases (Russo et al., 2012), suppressive effect on vascular endothelial growth factor (VEGF)-induced angiogenesis (Tian et al., 2014), preventing metastatic progression in breast cancer cells (Lirdprapamongkol et al., 2013), inhibition of TNF-α and interleukin (IL)-1β (Bai et al., 2013), anti-hypercholesterolemic activity (Anandhi et al., 2013), cardioprotective activity *via* improving post-ischaemic functional recovery (Testai et al., 2013), prevention of osteoporosis by activation of estrogen receptor (ER)/mitogen activated protein kinase (MAPK) (Zeng et al., 2013), and renoprotective activity by glucose-induced renal tubular cell migration with diminishing matrix metalloproteinase (MMP)-2 activity (Kang et al., 2015). In addition to all these pharmacological effectiveness of chrysin, it has also been shown to possess neuroprotective activity acting through various mechanisms. However, unlike other flavonoids, the therapeutic benefits of chrysin remains nascent in current literature due to issues with bioavailability and absorption. Consequently, the target of the current review is to articulate neurological potential of chrysin particularly referring to neuroinflammation, antidepressant, anti-epileptic, anti-amyloidogenic, anti-atherogenic effects as well as its chemistry and bioavailability.

2. Chemistry & occurrence

Chrysin (5,7-dihydroxyflavone or 5,7-dihydroxy-2-phenyl-4H-chromen-4-one) belongs to the flavone class of the ubiquitous 15-carbon skeleton natural polyphenolic compounds collectively called flavonoids. The characteristic feature of flavones as evidenced in chrysin is the presence C2-C3 double bond in ring C and the lack of oxygenation at C-3 (Fig. 1). Unlike many flavonoids that possess either one (most commonly at C-4′) or two hydroxy (C3′,C4′-diortho hydroxyl) functional group in ring-B, chrysin lacks oxygenation in this ring. Other natural derivatives of chrysin arise due to diversity in ring-A oxygenation as exemplified by the common natural biologically important flavonoids such as wogonin, oroxylin A and baicalein (Fig. 1).

Fruits and vegetables are the main dietary sources of flavonoids. Due to the presence of these compounds, health benefits are ascribed to vegetable foods when they are consumed on regular basis. Flavonoids are also present in many medicinal plants and account for the different pharmacological benefits reported to date. Chrysin and its derivatives have been shown to be the principal constituents of the well-known medicinal plant, *Radix scutellariae* (Tong et al., 2012). Other common sources of chrysin, which attracted much scientific attention in recent years from the pharmacological point of view, are propolis and/or honey (Bertoncelj et al., 2011; Sobočanec et al., 2006; Volpi and Bergonzini, 2006). Moreover, many fruits (Chen et al., 2014), passion flowers such as *P. caerulea* L (Wolfman et al., 1994) and even mushrooms (e.g. oyster mushroom, *Pleurotus ostreatus*; Anandhi et al., 2013) are known to be good sources of chrysin. While chrysin-containing plants have been widely used for medicinal purposes, synthetic chrysin is used for large scale uses. The range of different methods for chrysin synthesis has been growing over recent years (e.g. Man et al. (Liu et al., 2014)) and gram quantities of this compound can be purchased from commercial sources at a reasonable price.

3. Structure-activity relationships

The chemical properties of chrysin, due to B and C-ring lack of oxygenation, are associated with a number of pharmacological activities that range from antioxidant to anticancer effects (Habtemariam, 1997). However, differences in the chemical structure of flavones has been shown to influence the antioxidant activity, and the inhibitory effect on the expression of the pro-inflammatory enzyme cyclooxygenase-2 (Cox-2). For instance, luteolin, an equally important flavone, demonstrated greater Cox-2 inhibition than chrysin (Harris et al., 2006). This has been attributed to chrysin's lack of 3′,4′ hydroxylation on the “B” ring. Ko et al. showed that 2′ or 4′ B ring hydroxylation was necessary for the inhibition of phorbol ester-induced Cox-2 expression by flavanones (Ko et al., 2002). Similarly, Hou et al. demonstrated that an ortho-hydroxyl group is a requirement for the inhibition of LPS-induced Cox-2 expression in RAW 264.7 cells by anthocyanins (Hou et al., 2005).

As well, luteolin, which possesses 3′,4′ hydroxylation exhibits

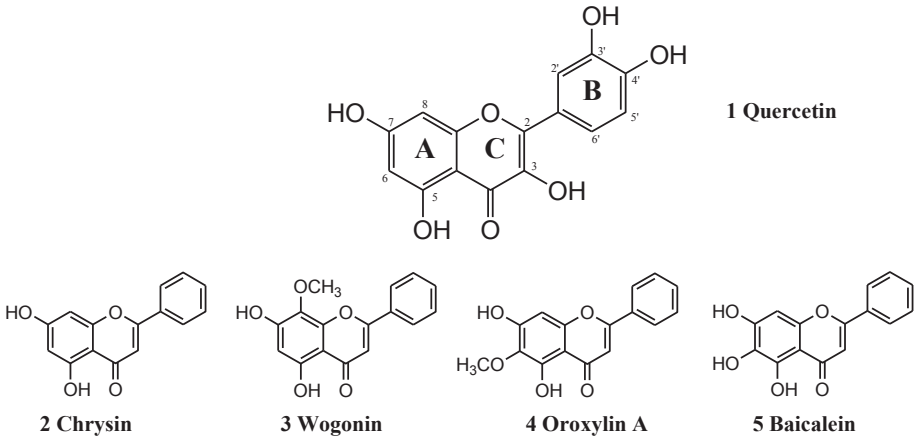

Fig. 1. The flavonoids skeleton. The common flavonoid rings and numbering is shown by using one of the most abundant flavonoid, quercetin (1). The structure of chrysin and some most structurally close natural derivatives are also shown.

greater antioxidant activity than chrysin. The presence of 3′,4′ hydroxylation, a double bond between carbons 2 and 3, and the presence of a carbonyl group on carbon 4 have been reported to be essential to produce antioxidant activity (Heim et al., 2002). Unlike chrysin, luteolin possesses all these structures and effectively scavenged the hydroxyl and superoxide radicals in RAW 264.7 macrophage-like cells at micromolar levels, whereas chrysin was unable to scavenge the superoxide radical, and scavenged the hydroxyl radical less effectively than luteolin at comparable doses (Harris et al., 2006). Since chrysin can inhibit the generation of the highly reactive hydroxyl radical, but to a lower extent than luteolin, B-ring hydroxylation (which is absent in chrysin) is not essential for scavenging of the hydroxyl radical.

In recent years, various attempts have been made through the synthesis of derivatives/analogues, to further increase its biological effects. By introducing various substituents shown in Fig. 2 the inhibitory activity against COX-2, prostaglandin E2 (PGE_2) and NO production was enhanced *in vitro* (Gao et al., 2013). Lim et al. (Lim et al., 2011) also demonstrated similar anti-inflammatory effect for compound **8** (Fig. 2) in LPS-treated RAW cells, where IC_{50} values were 6.2 and 22.6 μM for COX-2 mediated PGE_2, and inducible nitric oxide (iNOS)-mediated NO production, respectively. In a similar study carried out by Dao et al. (Dao et al., 2004), several derivatives of chrysin were synthesised. The 3′,4′-dichloro substituent appears to display good inhibitory activity against prostaglandin production

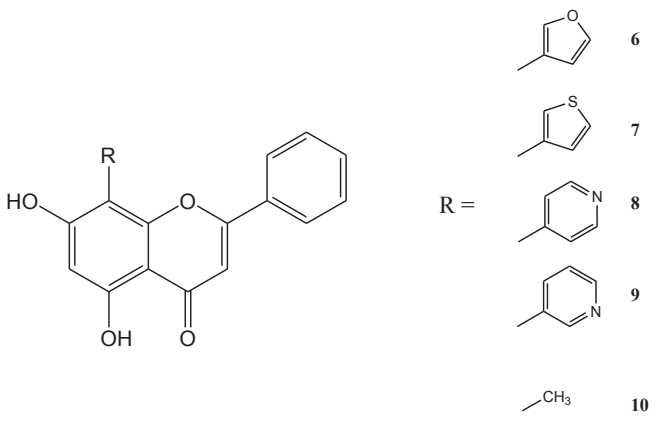

Fig. 2. Some derivatives of chrysin with *in vitro* antiinflammatory effect. Significant PGE_2 and NO production were demonstrated when tested at 10 μM concentration (Che et al., 2011).

as over 95% inhibition was recorded at 10 μM concentration. Another approach aimed to enhance the anti-inflammatory activity was developed by Wang et al. (Wang et al., 2014). In this research, the incorporation of hydrophobic chains at the C-5 and C-7 hydroxy positions of chrysin was studied. Various chain lengths of nitric oxide donors $-O(CH_2)_nONO_2$ and $-OCH_2COO(CH_2)_nONO_2$ were introduced and this led to a significant α-glucosidase inhibition. The approach of nitric oxide donor pro-drugs using chrysin skeleton has also been suggested to be a valid route for obtaining potential vasculoprotective agents (Zou et al., 2010) and angiogenesis promoters (Peng et al., 2009).

Another interesting derivative of chrysin was compound **11** (Fig. 3) (Lv et al., 2011), which was shown to display immunosuppressive inhibitory activity with IC_{50} of 0.78 μM, comparable to cyclosporin A (IC_{50} = 0.06 μM).

With the expectation of obtaining potent antiproliferative chrysin derivatives, Park et al. (Park et al., 2005) synthesised a series of long-chain derivatives of (**12**) (Fig. 4). Two compounds of this series, *hexadecyl 2-(5-hydroxy-4-oxo-2-phenyl-4H-chromen-7-yloxy)acetate* and *N-hexadecyl 2-(5-hydroxy-4-oxo-2-phenyl-4H-chromen-7-yloxy)acetamide*, were found to display promising activity. An EGFR inhibitory activity (IC_{50} values of 0.048 μM and 0.035 μM) comparable to the positive control erlotinib coupled with antiprolifereative effect against human liver cancer cell line HT-29 were reported. Although the reported activity was weak (IC_{50} value of 141 μM), Zhu et al. (Zhu et al., 2014) have also shown that 5,7-diacetyl chrysin displayed enhanced antitumour activity in H22 cells *in vitro*. Phosphorylation at the C-7 and preferably both C-5 and C-7 positions as exemplified by diethyl chrysin-7-yl phosphate and tetraethyl bis-phosphoric ester of chrysin similarly enhanced the antiproliferative effect of chrysin (Zhang et al., 2004).

Furthermore, Shin et al. (Shin et al., 1999) have shown that simple propyl, butyl, octyl and tolyl derivatives of the 5- and 7-

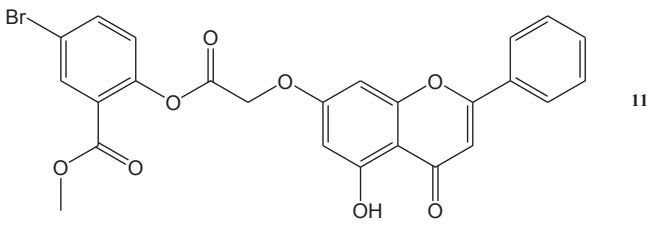

Fig. 3. Derivative of chrysin with immunosuppressive inhibitory activity.

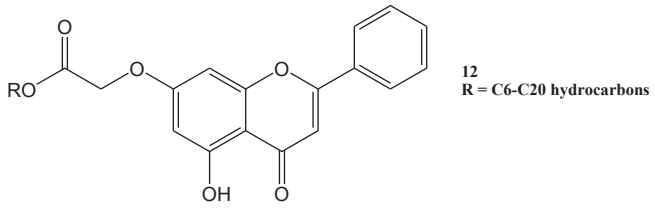

Fig. 4. Chemical structure of antiproliferative chrysin derivatives.

hydroxyl groups were found to show antiglycemic effect without toxicity to animals up to 500 mg/kg. Interestingly, chrysin itself inhibited insulin release by 40–60% in the same model (Shin et al., 1999).

4. Bioavailability and toxicity

The beneficial effects of chrysin and other naturally occurring phytochemicals is dependent on their bioavailability and achievable concentration *in vivo*, which is in turn, dependent on the solubility of these compounds. One study reported in the plasma concentrations of unchanged chrysin following a single 400 mg oral dose of this flavonoid to be very low in seven healthy human volunteers (Walle et al., 2001). The plasma binding of chrysin was estimated to be >99%. This reported value is very similar to that reported for the flavonoid quercetin. The volume of distribution for quercetin is also quite low (2–18 l) (Boulton et al., 1998). This effect is likely due to its extensive plasma binding. Using this value of volume of distribution, Walle et al. reported that the oral bioavailability of chrysin was 0.003–0.02% (Walle et al., 2001). The maximum concentrations of chrysin in plasma are 12–64 nM (Kao et al., 1998). Although serum concentrations have not been reported for chrysin, the estimated maximum serum concentration for flavonoid aglycones in general is 1 μmol/L (Walle et al., 2001). Therefore, treatment with chrysin is in the micromolar range.

An interesting array of studies using the human colonic cell line Caco-2 as a model of intestinal absorption has concluded that chrysin has favourable membrane transport properties but its intestinal absorption is limited due to efficient glucuronidation and sulfation in these cells (Walle et al., 1999b). The elimination of chrysin metabolites may depend on efflux by the MRP2 transporter. After efflux into the intestine these conjugates may be hydrolysed by sulphatases and glucuronidases to chrysin, as observed in the stool samples. However, the appearance of large amounts of unchanged chrysin in the stool samples suggest that chrysin has poor intestinal absorption (Walle et al., 1999a). Hence, derivatives of chrysin and further formulation studies are essential for establishing the overall therapeutic potential of chrysin and its analogues. Some preliminary studies on formulation such as inclusion in β-cyclodextrin nanocavity has been shown a promise (Chakraborty et al., 2010) but more comprehensive data is needed in this field.

Although low doses of flavonoids, which are present in our daily diet, may be safe for human consumption, ingestion of higher doses may lead to toxicity. The simple flavone chrysin, can be purchased from health food stores and through the Internet at "recommended daily doses" of 0.5–3 g. However, chrysin has been shown to induce toxicity in trout liver cells (Tsuji and Walle, 2008). More specifically, chrysin treatment inhibited *de novo* DNA synthesis leading to reduced cell numbers. The IC_{50} values were as low as about 2 μM, which is a concentration that is 25 times less than that producing antiproliferative effects in a number of cell lines (Tsuji and Walle, 2008).

The cytotoxicity due to chrysin has been attributed to the presence of peroxidase-like activity in hepatocytes, leading to oxidation of chrysin to form toxic products (Tsuji and Walle, 2008). Myeloperoxidase in neutrophils has been previously associated with drug-induced toxicities, such as agranulocytosis and lupus reported by carbamazepine (Gardner et al., 2005) and clozapine (Gardner et al., 1998) and the topoisomerase II poison etoposide (Kagan et al., 2001). It is likely that this enzyme may also be responsible for the toxicity induced by dietary flavonoids.

5. Neuroprotective effects of chrysin

5.1. Neuroinflammation

It is well established that activation of microglia is a major inducer of neuroinflammation in almost all neurodegenerative diseases, and Alzheimer's and Parkinson's disease in particular (Elmore et al., 2015; Madeira et al., 2015). Therefore, suppression of microglial activation represents a major target to enhance neuronal cell survival. One study recently showed that chrysin treatment significantly inhibited the release of nitric oxide (NO) and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in lipopolysaccharide (LPS)-stimulated microglia. As well, the expressions of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) were also significantly inhibited by chrysin. Importantly, chrysin was also shown to inhibit the activations of c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB), which are key mediators of neuroinflammation (Ha et al., 2010). However, another study showed that chrysin treatment (0.1–1 μM) for 18 h could not modulate constitutive or TNFα-induced NF-κB activity in primary cultures of mouse cortical astrocytes (Spilsbury et al., 2012). Therefore, it is likely that NF-κB signalling in astrocytes is not a target for chrysin.

Additionally, chrysin pre-treatment inhibited nitric oxide and TNF-α production, and iNOS expression in both LPS and interferon-γ-stimulated microglial cells. Contrary to the previous study, no effect was reported on the expression of the proinflammatory enzyme, cyclooxygenase-2 (Gresa-Arribas et al., 2010). Chrysin pretreatment also attenuated neurotoxicity due to microglial activation in primary murine neurons. The neuroprotective effects of chrysin were accompanied by decreases in the expression of CCAAT/enhancer binding proteins (C/EBPs) β and δ transcription factors at both the mRNA and protein level, and DNA binding activity (Gresa-Arribas et al., 2010). This suggests that C/EBPδ may mediate the anti-inflammatory effects of chrysin in brain cells.

Neuroinflammation also plays a major role in the pathogenesis of focal cerebral ischemia/reperfusion (I/R) injury, culminating in neuronal cell death. Using a mouse model of middle cerebral artery occlusion (MCAO), male C57/BL6 mice were pretreated with chrysin once a day for seven days (Yao et al., 2014). Afterwards, subjects were then subjected to 1 h of middle cerebral artery occlusion followed by reperfusion for 24 h. Chrysin treatment significantly attenuated the increases in glial cell numbers and secretion of proinflammatory cytokines occurring due to ischemia/reperfusion. As well, chrysin also inhibited the MCAO-induced upregulation of nuclear factor-kappa B (NF-κB), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) compared to the vehicle group. The biochemical changes occurred parallel to a significant decline in neurological deficit scores and infarct volumes, compared with the vehicle group (He et al., 2012; Yao et al., 2014). This suggests that chrysin may represent a promising antidote to restore normal cellular homeostasis following I/R injury.

5.2. Antioxidant and neurotrophic effects

Chrysin has also been shown to protect against hydrogen

peroxide-induced apoptosis and attenuate neuronal death in several *in vitro* models (Izuta et al., 2008; Kang et al., 2004). Chrysin has also been shown to slow down age-related increased in oxidative stress and improve cognitive decline and reductions in brain-derived neurotrophic factor (BDNF) in mice *in vivo* (Souza et al., 2015). Oral administration of chrysin at doses of 1 mg/kg and 10 mg/kg significantly attenuated the increase in free radical production, and inhibited the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and Na(+), K(+)-ATPase levels and the inhibition of SOD, CAT and GPx activities of aged mice. Chrysin modifies Na(+), K(+)-ATPase activity in the prefrontal cortex and hippocampus of mice. Chrysin treatment also mitigated the reduction in BDNF levels in the prefrontal cortex and hippocampus of aged mice. Similarly, age-related memory decline was partially protected by chrysin at a dose of 1 mg/kg, and normalized at the dose of 10 mg/kg using the Morris Water Maze task (Souza et al., 2015). Taken together, these data suggest that chrysin can prevent age-related decline in memory due to its potent antioxidant effects and modulation of BDNF production.

5.3. Anti-depressant effects

Alterations in brain neurotrophins and brain Na(+), K(+)-ATPase have been strongly associated with the development of depression in experimental animals. The therapeutic role of chrysin as antidepressant compound has been previously assessed using animals subjected to chronic unpredictable mild stress (CUMS) (Dubey et al., 2015; Filho et al., 2015). The study showed that CUMS applied for 28 days to female mice induced a significant decline in BDNF and nerve growth factor (NGF) levels, and Na(+), K(+)-ATPase activity (Filho et al., 2015). CUMS also enhanced the development of a depressive status in the forced swimming test (FST), and sucrose preference test, with significant elevations in corticosterone levels. Oral treatment with chrysin (20 mg/kg) attenuated the decrease in BDNF and NGF levels in mice subjected to CUMS, comparable to fluoxetine. Chrysin also attenuated the increase in glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT) activities in mice exposed to CUMS (Filho et al., 2015). This suggests that the upregulation of BDNF and NGF, together with the potent antioxidant function of chrysin, is the primary mechanism to explain the anti-depressant effect of chrysin *in vivo*.

5.4. Anti-atherogenic effects

Hyperlipidemia has been linked to the pathogenesis of a several neurological conditions, including Alzheimer's disease (Deckers et al., 2015). Oxidative stress is thought to contribute to the development of hyperlipidemia. The anti-atherogenic and antioxidant efficacy of chrysin, was previously evaluated in an experimental model of atherosclerosis (Anandhi et al., 2014). Atherogenic diet-fed male albino Wistar rats, receiving oral chrysin treatment (200 mg/kg b.wt) for 15 days, starting 30 days after the start of the atherogenic diet, showed significantly lower mean serum levels of lipid profile parameters (except for HDL-cholesterol which was elevated), hepatic marker enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase), and significantly higher mean hepatic levels of lipoprotein lipase (LPL), 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA reductase), enzymatic (catalase, superoxide dismutase, and glutathione peroxidase), and non-enzymatic antioxidants (reduced glutathione, and vitamins C and E)and significantly lower mean levels of hepatic malondialdehyde, compared to the rats exposed to an atherogenic diet alone. Histopathological, chrysin reduced hepatic damage and size of atherosclerotic plaques in the aorta of atherosclerotic rats (Anandhi et al., 2014). This is the first study to suggest that chrysin exhibits anti-atherogenic properties *in vivo*.

5.5. Anti-amyloidogenic effects

Aggregation of the amyloid beta protein (Aβ) has been proposed as one of the likely causes of the progressive loss of cholinergic neurons in Alzheimer's disease (Balakrishnan et al., 2015). The structural requirements for the anti-amyloidogenic activity of several structurally related flavonoids on Aβ fibril formation has been investigated *in vitro* (Akaishi et al., 2008; Zhu et al., 2007). $A\beta_{1-42}$ (20 μM) and the flavonoids were incubated for 48 h at 37 °C, and fibril formation was quantified using the thioflavin T fluorescence assay. Flavonoids such as fisetin, 3′,4′,7-trihydroxyflavone, 3,3′,4'-trihydroxyflavone, luteolin, quercetin and myricetin, which contain the 3′,4'-dihydroxyl group inhibited Aβ fibril formation. However, flavonoids including 5-deoxykaempferol, and chrysin (5,7-hydroxyflavone) enhanced the formation of Aβ fibrils (Akaishi et al., 2008). This suggests that the 3′,4′-dihydroxyl group, but not the 3- or 7-hydroxyl group is required for the inhibitory effect on Aβ fibril formation.

5.6. Effects on Parkinson's disease

The neuroprotective effects of several members of the flavonoid family against oxidative insult to mesencephalic dopamine (DA) neurones following exposure to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPP+) has been previously investigated. DNA fragmentation studies and tyrosine hydroxylase (TH) immunocytochemistry of DA neurones were used to demonstrate that chrysin, along with other flavonoids, including catechin, quercetin, puerarin, naringenin, and genistein could protect mesencephalic cultures from injury by MPP+ (Mercer et al., 2005). Since MPP+ neurotoxicity is an important *in vitro* model for the identification of neuroprotective agents, these DA findings suggest that chrysin and a number of other flavonoids may be beneficial for the treatment of Parkinson's disease (Mercer et al., 2005).

The combined neuroprotective effects of chrysin and another polyphenol, protocatechuic acid (PCA) has also been examined. The study reported synergistic neuroprotective effects, with chrysin enhancing the protective effects of PCA, leading to greater cell viability and reduced lactate dehydrogenase release from PC12 cells treated 6-hydroxydopamine at pathophysiological levels (Zhang et al., 2015). Combined treatment with both chrysin and PCA also significantly attenuated chemically induced dopaminergic neuronal loss in both zebrafish and mice. The mechanism of action for the observed neuroprotective effects following pretreatment with chrysin and PCA is attributed to: (i) increased nuclear factor-erythroid 2-related factor 2 protein expression and transcriptional activity; (ii) modulation of cellular redox status with upregulation of endogenous antioxidant enzymes, including heme oxygenase-1, superoxide dismutase, and catalase; (iii) reduced levels of malondialdehyde, a known marker for lipid peroxidation, and (iv) inhibition of nuclear factor-κB activation and down-regulation of inducible nitric oxide synthase expression (Zhang et al., 2015, 2012). Therefore, a therapy combining both chrysin and PCA may facilitate neuroprotection through a combination of antioxidant and anti-inflammatory mechanisms.

5.7. Anti-epileptic effects

Apart from its potent antioxidant and anti-inflammatory effects, chrysin has also been shown to be a ligand for the benzodiazepine receptors, both central (Ki = 3 microM, competitive mechanism)

and peripheral (Ki = 13 microM, mixed-type mechanism) (Medina et al., 1990). Intracerebroventricular administration of chrysin in mice has been shown to ameliorate the expression of tonic-clonic seizures induced by pentylenetertrazol. The normal righting reflex was restored in all chrysin-treated mice, suggesting a myorelaxant effect of the flavonoid (Medina et al., 1990). Another study showed that mice treated with extracts of *Passiflora edulis* Sims (accepted name of *P. incarnata* L.) (where chrysin is the main constituent) demonstrated significantly reduced seizure severity and immobility period compared to vehicle control in a dose and time-dependent manner after 15 days treatment (Singh et al., 2012). Moreover, extract treatment retained the serotonin and noradrenaline levels in the brain (Singh et al., 2012). This suggests that plant extracts containing chrysin as the main bioactive component may be used as an adjunct to standard diazepam treatment, which worsens post-ictal depression (Sampath et al., 2011).

5.8. Protective effects against spinal cord injury

The neuroprotective efficacy of chrysin has also been evaluated in an experimental rat model of spinal cord injury (SCI). Chronic treatment with chrysin (20 and 40 mg/kg p.o.) significantly and dose-dependently ($p < 0.05$) attenuated the decline in body weight, urine output, footprint analysis, sperm count and organ weight (testis, seminal vesicle and urinary bladder) in male Sprague–Dawley rats where SCI was induced by placing an aneurysm clip extradurally for 60 s at T10 (Kandhare et al., 2014). Significant improvements in the nociceptive threshold, motor and sensory nerve conduction velocity where also reported following chrysin treatment. Apart from behavioural effects, chrysin treatment also decreased the activity of superoxide dismutase and lipid peroxidase, lowered the levels of nitric oxide, tumor necrosis factor alpha, and interleukin-1β, and reduced the expression of bax, bcl-2 and caspase-3. Other biochemical changes due to chrysin treatment included reduced glutathione and membrane-bound inorganic phosphate (Kandhare et al., 2014). Another study showed reduced inflammatory responses and impaired nitric oxide synthase pathway following SCI in rats treated with chrysin (Jiang et al., 2014). Therefore, it is likely that chrysin can enhance recovery of both motor and sensory functions *via* modulation of endogenous biomarkers and neuronal apoptosis to attenuate the development neurological deficits due to SCI.

6. Conclusion and recommendations

Epidemiological studies demonstrated that elderly people will increase over the coming decades. Unfortunately, this increase is associated with the increase of age-related diseases, especially mental diseases. Therefore, recent research has been focused on the discovery of neuroprotective agents with high efficacy and negligible adverse effects. Polyphenols could be considered interesting target for drug design and discovery due to the growing evidence that suggest that flavonoids possess beneficial effects on mental diseases. Chrysin is an important natural neuroprotective agent which is widely found in different fruits and vegetables as well as mushrooms. In this paper we demonstrated that chrysin mitigates neurotoxicity and oxidative stress in the neural tissues. In addition, we showed that chrysin mitigates epilepsy, neuroinflammation as well as cognitive dysfunctions. Moreover, chrysin possesses potent antidepressant, anti-amyloidogenic and neurotrophic effects. Despite to these, our search in clinicaltrials.gov database with keyword “chrysin” showed that there is lack of clinical studies regarding to neuroprotective effects of chrysin. Thus, it can be difficult to make a clear decision about its clinical impacts. We suggested that future studies should focus on:

- Bioavailability, pharmacokinetic and pharmacodynamics of chrysin and its derivatives.
- Molecular mechanisms underlying its neuroprotective effects.
- Toxicity studies, which are required for its clinical use.
- Clinical trials aimed to evaluate its beneficial effects on brain diseases.

Conflicts of interest

The authors have declared none.

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