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Review

Potential involvement of oxidative stress in induction of neurodegenerative diseases: Actions, mechanisms and neurotherapeutic potential of natural antioxidants

George Laylson da Silva Oliveira¹, Francisco Rodrigo de Asevedo Mendes de Oliveira¹ and Rivelilson Mendes de Freitas¹*

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Several studies have shown that oxidative damage to various organs of a given organism is involved in causing various diseases, and in this context, the brain is highly vulnerable to oxidative stress because of its high metabolic rate, low capacity of cell regeneration, small amounts of enzymatic and non-enzymatic antioxidants. Oxidative stress is caused by an imbalance in cell redox state, in which there is excessive production of reactive oxygen species and/or failure of antioxidant defense systems. Consequently, oxidative stress in brain has shown direct implications in the pathogenesis of several neuro-degenerative diseases such as Alzheimer’s, Parkinson’s, Huntington’s, Schizophrenia and amyotrophic lateral sclerosis. In this review, the critical role of oxidative stress in neuro-degenerative diseases as well as some aspects of antioxidant compounds (phenolic compounds and vitamins) regarding prevention and/or treatment of Alzheimer’s, Parkinson’s, Huntington’s, Schizophrenia and amyotrophic lateral sclerosis were evaluated.

Key words: Natural antioxidants, brain, neurodegenerative diseases, oxidative stress.

INTRODUCTION

Oxidative stress causes damage to various cellular processes in a deleterious manner, and consequently plays important roles in the development of many diseases, including those that affect the central nervous system such as neuro-degenerative diseases. Thus, many studies have aimed at researching new treatment strategies using antioxidants as a therapy for neuro-degenerative diseases (Choi et al., 2012; Johri and Beal, 2012; Jin et al., 2013a; Li et al., 2014; Deslauriers et al., 2014). This review reports a general view on oxidative stress in pathophysiology of neuro-degenerative diseases and also discussed the use of antioxidants of natural origin as neurotherapy in diseases such as Alzheimer’s, Parkinson’s, Huntington’s, Schizophrenia and amyotrophic lateral sclerosis.

This review was made based on a literature search using Science Direct (http://www.sciencedirect.com), Scopus (http://www.scopus.com), Pub Med (http://www.ncbi.nlm.nih.gov/pubmed), Web of Science (http://wokinfo.com) and SciFinder (http://cas.org/products/scifindr/index.html). The search was conducted until May 2014 using the following terms:

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Oxidative stress, reactive oxygen species, reactive nitrogen species, brain, antioxidants, neurodegenerative diseases, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Schizophrenia, Huntington's disease, phenolic compounds and vitamins. These words were also used in various combinations, being considered valid original research article, full text and that provided the search terms in the title and/or abstract. The duplicate articles in scientific databases were excluded and the remaining ones were evaluated by their eligibility regarding the inclusion and exclusion criteria, by reading and analyzing title and abstract. Articles in English published up to 2014 were analyzed.

OXIDATIVE STRESS IN BRAIN

The chemical substances that have one or more unpaired electrons are considered free radicals, which have as main characteristic that facility to donate their electrons to other molecules causing chain reactions and oxidative damage (Liochev, 2013). Free radicals and related molecules are classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and a wide variety of these radicals are produced during normal metabolism in biological systems, which are counter balanced by cellular antioxidant mechanisms. However, the imbalance by excessive ROS and RNS and decreased antioxidant defense systems at cellular level cause oxidative stress (Figure 1), which can be induced to damage by peroxidation of cellular structures, protein oxidation, DNA damage and inhibition of electron transport chain in mitochondria (Dasuri et al., 2013).

ROS are represented by a group of chemicals that include the superoxide anion (O$_2^-$), hydroxyl (HO$^-$), peroxyl (RO$_2^-$), hydroperoxyl (HO$_2^-$), lipid hydroperoxide (LOOH), hypochlorous acid (HOCl), singlet oxygen (O$_2^*$) and hydrogen peroxide (H$_2$O$_2$). RNS produced in cells are represented by nitric oxide (NO) and peroxynitrite (ONOO$^-$) (Roberts et al., 2010; Koskenkorva-Frank et al., 2013). The superoxide anion, hydroxyl and hydrogen peroxide are the main ROS. Among these, the superoxide anion is formed due to reduction of molecular oxygen by various enzymatic systems (enzymes in the electron transport chain in mitochondria, xanthine oxidase, cyclooxygenase and NADPH-oxidase). In turn, the enzyme superoxide dismutase (SOD) converts the superoxide anion into molecules of hydrogen peroxide. Hydrogen peroxide in other reactions, in the presence of transition metals (iron and copper) is converted into a very reactive ROS, the hydroxyl radical (Milenkovic et al., 2013; Koskenkorva-Frank et al., 2013). One of the main mechanisms responsible by production of ROS in cells of the brain tissue is illustrated in Figure 2.

Several studies have considered that oxidative damage to various organs of a given organism is involved in the onset of various diseases. In this context, the brain is an organ highly vulnerable to oxidative stress due to its high metabolic rate, which accounts for 20% of oxygen consumption (Sultana et al., 2013). In addition, the brain has low capacity for cellular regeneration, small amount of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase) and antioxidant non-enzymatic (reduced glutathione, α-tocopherol and ascorbic acid) and as in any other tissue, oxidative stress in brain can damage the neurons by peroxidation of poly-unsaturated fatty acids (arachidonic acid and docosahexaenoic acid) in cell membrane, oxidative damage to DNA, RNA, proteins and induction of apoptosis (Figure 3) (Nunomura et al., 2012; Smith et al., 2013).

 Peroxidation of the cell membrane has as consequence the increase of stiffness, decreased activity of membrane bound enzymes, deficiency of membrane receptors and alters the permeability. ROS and RNS can also attack directly the proteins of the lipid membrane and cause fragmentation of proteins, cross-links between proteins and the formation of carbonyl, which contributes to the integrity of the cell membrane and interferes with various cellular processes. ROS and RNS can still attack the nucleic acids causing the crosslinking of the protein-DNA, modification of DNA bases, breakage and mutations in the DNA chain (Sultana et al., 2013).

OXIDATIVE STRESS AND NEURODEGENERATIVE DISEASES

Oxidative stress and Alzheimer's diseases

Alzheimer's disease (AD) is clinically characterized by progressive memory loss and severe cognitive function decline. The accumulation of extracellular amyloid protein deposited senile plaques and intracellular neurofibrillary tangles made of abnormal and hyper-phosphorylated tau protein, regionalized neuronal death, loss of synaptic connections in selective brain regions, proliferation of astrocytes and microglia activation, and define neuro-pathologically the AD. In addition, the oxidative stress and mitochondrial dysfunction are related to the development of AD (Gubandru et al., 2013).

Mitochondria are responsible for ATP production, oxidative phosphorylation, cell cycle control, cell growth and apoptosis (Chaturvedi and Beal, 2013). Thus, a problem in energy metabolism contributes to reduced production of ATP, beyond the generation of excessive ROS, such as superoxide, hydroxyl radicals and hydrogen peroxide. The literature describes the mitochondrial damage induced by ROS and vascular hyperfusion as key initiators for the development of AD (Butterfield et al., 2006; Aliev et al., 2010; Massaad et al., 2009; Parihar and Brewer, 2007; Kovacic and Somanathan, 2012; Schrag et al., 2013).

Indeed, oxidative imbalance and significant increase of its bio-products have been consistently reported in AD. Thus, the products generated from oxidized biomolecules
Free radicals
$O_2^{•-}, H_2O_2, OH^{•}, NO^{•}, HOCl, ONOO^{-}$

Enzymatic and non-enzymatic antioxidant defense system
SOD, CAT, GPx, GSH, ascorbate, $α$-tocopherol

**Figure 1.** Representation of oxidative stress.

**Figure 2.** Representation of ROS/RNS in cells of nervous tissue by reduction of molecular oxygen to water in mitochondrial electron transport chain, which consists of four multimeric complexes (I, II, III and IV) and two conveyors of electrons (Coenzyme Q and Cytochrome C). Complexes I and III are the major sources of production of free radicals.
are stable and used as markers of ROS. Moreover, exogenous antioxidants and antioxidant enzymes can indirectly measure the levels of ROS. In that sense, in lipid peroxidation, significant levels of reactive aldehydes may be evidenced, including 4-hydroxynonal, malondialdehyde (MDA), and 2-propanal (acrolein) and are chemically and metabolically stable iso-prostanoids including F2-isoprostanes and F4-neuroprostanes (Wang et al., 2013a; Gubandru et al., 2013; Sultana et al., 2013). Several investigations show that in the oxidation of proteins, the most investigated markers are carbonyls and 3-nitrotyrosine, final product of the interaction of peroxy nitrite with tyrosine residues (Aksenov et al., 2001; Castegna et al., 2002; Good et al., 1996; Tohgi et al., 1999; Butterfield et al., 2001; Castegna et al., 2003; Reed et al., 2009).

In the oxidation of the genetic material (DNA/RNA), the hydroxyl deoxyguanosine 8-(8-OHdG) and 8-hydroxyguanosine (8-OHG) are the main markers, which are the products of oxidation of guanine (Mecocci et al., 1994; Nunomura et al., 1999; Shan et al., 2003; Ding et al., 2005; Honda et al., 2005). Besides the increase these toxic products of biomolecules, significant reduction of antioxidants and antioxidant enzymes can be verified. Study reveals that plasmatic levels of antioxidants such as albumin, bilirubin, uric acid, lycopene, vitamin A, vitamin C and vitamin E are decreased in patients with AD. Similar results can be shown with antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and hemeoxigenase, in different brain regions (Wang et al., 2013; Foy et al., 1999; Kim et al., 2006). Despite continued efforts, current therapeutic strategies are limited to those that soften the symptoms without impeding the progress of the disease. Thus, there

Figure 3. Oxidative damage caused by ROS and RNS to various biomolecules.
is growing demand for a treatment capable of protecting neurons and inhibiting oxidative damage, may be an effective alternative evidenced in natural antioxidants (Bonda et al., 2010).

Oxidative stress and Parkinson's diseases

Parkinson's disease (PD) is a chronic neurological progressive disease and associated with a loss of dopaminergic neurons in the compact part of the substantia nigra (SN), clinically characterized by cardinal symptoms, resting tremor, rigidity, brady kinesia and postural instability. In addition to dopamine depletion, PD is neuropathologically defined by the deposition of Lewis bodies and Lewis neurites in vulnerable neurons. The etio-pathogenesis of PD is still not fully understood. In most cases the disease is eventual: a multifactorial idiopathic disease, seems to arise from a combination of genetic susceptibility and environmental exposures (Perfeito et al., 2013; Dickson et al., 2009; Halliday et al., 2011; Hauser and Hastings, 2013).

Some environmental toxins are involved in the process of oxidative stress, including paraquat, rotenone, maneb and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The MPTP, for example, crosses the blood-brain barrier due to their lipophilicity and is oxidized to a toxic substance, MPP+ (1-methyl-4-phenylpyridinium) by monoamine oxidase in glial cells. MPP+ is accumulated in mitochondria of dopaminergic neurons. The MPP+ inhibits mitochondrial complex I, causing break in electron transport chain and as a result the reduction in ATP production and increased production of ROS (Subramaniam and Chesselet, 2013; Chen and Le, 2006).

Moreover, evidence shows an association between oxidative stress and mitochondrial DNA (mtDNA) mutations with the onset of PD. Associated with constant exposure to ROS, the lack of protection by histones, and limited DNA repair mechanisms, make mtDNA particularly vulnerable to oxidative damage, which can lead to harmful mutations. As a result, a positive feedback is generated by the accumulation of these mutations, which can gradually cause a reduction in the efficiency of the electron transport chain (ETC) stimulating decreased ATP production and increased production of ROS, followed by eventual cell death (Yan et al., 2013; Sanders and Greenamyre, 2013).

In addition, ROS can irreversibly damage protein, for example, amino groups in side chains of specific amino acids can be modified to carbonyl groups (Greenamyre and Sanders, 2013). It recently shows an association between elevated levels of protein carbonyls and increased oxidative stress (Hyun et al., 2002). Another study showed damage of mitochondrial complex I subunit in PD brains related to high levels of protein carbonyl (Keeney et al., 2006).

Similarly, the oxidized lipids can exercise deleterious effects on neuronal function and lead to PD. In general, lipids involved in membrane fluidity and permeability, can store energy that participate in inflammatory processes and in apoptosis signaling. The poly-unsaturated fatty acids are more prone to lipid peroxidation, particularly in the brain there is a large amount of two of these, the arachidonic acid and docosahexaenoic acid. After the adipose tissue, the organ with the highest lipid content is the brain and thus, it is very prone to lipid peroxidation (Ruipérez et al., 2010; Bochkov et al., 2002; Roberts II and Fessel, 2004; Chen et al., 2008). Evidence supports an association between lipid peroxidation and PD (Hyun et al., 2002; Hoepken et al., 2007; Lee et al., 2001).

Oxidative stress and Huntington's diseases

Huntington disease (HD) is a progressive neuro-degenerative disorder characterized by presence of emotional and movement disorders and dementia (Ayala-Peña, 2013). HD has autosomal dominant transmission with the mutant gene termed IT15, responsible for the disease located on chromosome 4. The mutation responsible by this disease consists in an excessive number of abnormal tri-nucleotide cytosine-adenine-guanosine (CAG). The mutant gene encodes a protein called huntingtin, which interacts with several proteins that are involved in transcription, cell signaling and intracellular transport. As a result of repeated CAG, may be polyglutamine expansion located near the N-terminus of protein molecule. The expanded poly-glutamine chains lead to fragmentation of protein, which tends to accumulate inside the neuron. The aggregation of protein fragments causes changes in neuronal functioning and possibly plays a role in neuronal death process (Goldberg et al., 1994; DiMauro and Schon, 2008; Harjes and Wanker, 2003; Cattaneo et al., 2005; Ha et al., 2012).

It is known that oxidative stress is mediated by increased ROS, including superoxide, hydrogen peroxide and hydroxyl radical. These ROS impair cell function by degrading proteins, lipids and nucleic acids and consequently, there is a vicious cycle of mitochondrial oxidative damage in Huntington's diseases. In context of mutant Huntington expression, the ROS overproduction causes accumulation and exhaustion of mtDNA damage and reduced mitochondrial bioenergetics. Simultaneously, the mutant Huntington affects mtDNA repair, avoiding the accumulation of human apurinic/apyrimidinic endonuclease (APE1) in mitochondria, which further contributes to mitochondrial damage. As a result, the mtDNA damage leads to exacerbated mitochondrial dysfunction and possible neuro-degenerative diseases. In addition, oxidative damage to nuclear DNA also contributes to mitochondrial dysfunction and contributes to activation of DNA repair processes that may lead to expansion of the CAG repeat (Mochel and Haller, 2011;
Johri and Beal, 2012; Ayala-Peña, 2013). Furthermore, oxidative modification of proteins (protein carbonyl) and lipids (malondialdehyde and 4-hydroxynonenal) are also increased in HD in the brain (Beal and Browne, 2006).

Relevant characteristics that support the relationship between oxidative stress and HD, can be evidenced by accumulation of several markers, such as lipofuscin derived from peroxidation of unsaturated fatty acids, 3-nitrotyrosine acids (nitrated protein) as well as 8-hydroxy-2-deoxyguanosine (OH8dG) in mtDNA (Dhillon and Fenech, 2013).

Oxidative stress and Schizophrenia

Schizophrenia is a chronic debilitating neurodegenerative disorder and is characterized by several positive symptoms such as delusions, hallucinations, disorganized speech and negative symptoms, including deficits in cognitive and social capacity and blunted affect (Yao and Keshavani, 2011). The initial pathophysiology of schizophrenia collaborates to increase the generation of reactive oxygen species in the brain (Bitanihirwe and Woo, 2011). In addition, genetic evidence demonstrates that decreased capacity of synthesizing antioxidant enzymes, such as glutathione (GSH) under conditions of oxidative stress favor the appearance of schizophrenia (Gysin et al., 2007; From et al., 2009). As previously discussed, excessive ROS cause damage to important macromolecules such as DNA, proteins and lipids. It can affect the genetic material by modifying gene expression, protein oxidation, making them non-functional and peroxidative damage in lipids, which results in damage to cell membrane and cell organelles (Wu et al., 2013). Additionally, dopamine, an auto-oxidizable neurotransmitter, has a dihydroquinone structure that can be oxidized by molecular oxygen to form hydrogen peroxide and o-quinone. Thus, the catecholamines oxidation combined to deficient antioxidant system in the brain, produces an excess of free radicals resulting in oxidative stress (Bošković et al., 2011).

Oxidative stress and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is one of the major neurodegenerative disorders regarding the neuromuscular junction. The disease is characterized by selective degeneration of upper motor neurons (corticospinal) and lower (spinal and bulbar), which results in pathological mis-communication between nerve and muscle (Pansarasa et al., 2013). The majority of ALS cases are of unknown etiology (sporadic). However, the identification of mutant genes that predispose to these disorders has provided the means to better understand its pathogenesis. It is known that this condition occurs in a change mutant gene that encodes Cu/Zn superoxide dismutase type 1 (SOD1), enzyme that removes free radicals and protects cells (motor neurons) from oxidative stress (Halter et al., 2010; Rosen et al., 1993). Studies have shown that SOD1 in its mutant version can cause mitochondrial dysfunction. The accumulation of SOD1 aggregates in membrane of mitochondria can cause blockage of proteins import, excessive ROS production and eventual cell death by apoptosis (Vijayvergiya et al., 2005; Mattiazi et al., 2002; Takeuchi et al., 2002).

Natural antioxidants with neurotherapeutic potential

Recently, natural antioxidants have gained great importance in treatment or prevention of diseases such as cancer, diabetes, cardiovascular and mainly neurodegenerative disorders. Scientific studies have already developed and discussed in preceding paragraphs demonstrate that the generation of ROS and RNS have direct implications in neurodegenerative diseases such as Alzheimer’s, Parkinson’s, amyotrophic lateral sclerosis and Huntington’s disease. Thus, there is great interest in studies aiming to investigate the therapeutic potential of natural antioxidants for preventing or controlling oxidative stress in neurodegenerative diseases, since it is known that brain tissue is vulnerable to oxidative damage by having abundant lipid content and relative scarcity of antioxidant enzymes when compared with other tissues (Figure 4).

Antioxidants such as vitamins, phenolic compounds and flavonoids have been extensively investigated as potential therapeutic agents in vitro and in vivo for prevention of neurodegenerative diseases (Ebrahimim and Schluesener, 2012; Choi et al., 2012). Most of these antioxidant compounds can be found in fruits, vegetables, plants or synthesized, and the antioxidant mechanisms can act by induction of gene expression of endogenous antioxidant defense systems (activation of nuclear factor kappa B, NF-kB), regulation of ROS and RNS, interaction with oxidative pathways and the capacity to chelate metal ions responsible for the formation of free radicals (Dajas, 2012) (Figure 5).

Vitamins

Vitamins C, A, E and B₆ shown in Figure 6, are substances with high capacity to remove ROS and RNS in vitro and in vivo studies, and therefore can act in prevention of oxidative stress and possible treatment for neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease (Table 1). For example, studies performed by Ahmed (2012) in mice provided important evidence that dietary supplementation with vitamins slows the progression of neurodegenerative diseases like Alzheimer’s.
Vitamins C and E are natural compounds well known for their high antioxidant capacity in \textit{in vitro} and \textit{in vivo} experimental models. Once there is an involvement of ROS and RNS in neurodegenerative diseases, vitamins C and E have been considered as potential therapeutic agents for neurodegenerative diseases such as Alzheimer’s, Parkinson’s, Huntington’s and amyotrophic lateral sclerosis (Heo et al., 2013). For example, Miyake et al. (2011) investigated the relationship between intake of antioxidant vitamins and risk of Parkinson’s disease in Japan using data from a case-control study of hospital-based multicenter. This study included 249 patients with Parkinson’s disease and results indicated that a higher intake of vitamin E might be associated with a reduced risk of Parkinson’s disease. In another epidemiological study involving 6 patients bearer of Alzheimer’s disease, Morris et al. (2005) demonstrated that ingestion of vitamin E could contribute to preventing the neurodegenerative
disease.
Even considering the pharmacological potential of Vitamin C, there are conflicting scientific studies on its potential in treatment of various neurodegenerative diseases, including Alzheimer's disease. For example, in the study of Arlt et al. (2012) involving the 12 patients with Alzheimer's disease, was observed that supplementation with vitamin C did not have significant effect on the prevention of Alzheimer's disease over 1 year. In another study involving 57 patients with Alzheimer's disease and treated with vitamin E, it has been observed that the oxidative stress was not inhibited when it was considering the oxidized glutathione levels (Lloret et al., 2009).

Vitamins addressed in Figure 6 are part of human diet and are considered substances with high antioxidant capacity. Thus, these antioxidant properties have attracted great attention for the treatment of neurodegenerative diseases, but studies to date are contradictory regarding the therapeutic potential for neurodegenerative diseases.

PHENOLIC COMPOUNDS

Phenolic compounds have one or more hydroxyl groups bonded to a benzene ring, being chemical constituents found in a wide variety of plants and which are represented by a variety of classes of compounds that can be divided according to their chemical structures. Among these classes, there are phenolic acids (hydroxybenzoic acids (C₆-C₁) and hydroxycinnamic acids (C₆-C₃), flavonoids (C₆-C₃-C₆, which includes anthocyanins, flavonols, flavones, flavanones and isoflavones), stilbenes (C₆-C₂-C₆), lignans (C₅-C₃-C₆-C₃) and curcuminoïds (C₃-C₆-C₃-C₁-C₆)) (Figure 7) (Cheynier et al., 2013).

Antioxidant capacity of phenolic compounds occurs primarily by elimination of free radicals, chelation capacity and modulation of enzymes, as well as their effects on cell signaling pathways and gene expression, being these mechanisms dependent on the chemical characteristics of the compounds (De Mello and Fasolo, 2014). Animal studies, clinical and epidemiological, support the critical role of phenolic compounds in the prevention and treatment of various diseases such as neurodegenerative disorders (Table 2).

Among the studies of phenolic compounds approached in Figure 7, quercetin (flavonoid) has been shown to be promising in treating various neurodegenerative diseases (Jazvinščak et al., 2012; Pandey et al., 2012; Denny Joseph and Muralidhara, 2013). In a model of induced Huntington's disease in rats, treatment with quercetin at a dose of 25 mg/kg was able to reverse the inhibition of mitochondrial electron transport chain, restore ATP levels, prevent the mitochondrial dysfunction by inhibiting oxidative stress and increase the SOD and CAT activities (Sandhir and Mehrotra, 2013). In other in vitro and in vivo studies, quercetin has demonstrated a role in therapeutic strategies to treatment of neurodegenerative diseases in clinical settings to provide neuroprotection related to suppression of oxidative stress, improvement in behavioral function, reduction in infarct volume, cerebral edema and cell injury. In contrast, some studies contradict the neuroprotective potential of quercetin and other flavonoids (Ossola et al., 2009; Huebbe et al., 2010).

In another study, Mansouri et al. (2013) examined the neuroprotective effect of gallic acid (phenolic acid) at a dose of 30 mg/kg (orally, once daily for 26 days) on cognitive changes and oxidative stress in the brain induced...
Figure 7. Chemical structures of classes of phenolic compounds.
Table 1. Main vitamins with therapeutic potential for the treatment of neurodegenerative diseases.

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<td>1</td>
<td>Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, Schizophrenia</td>
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<td>Ballaz et al. (2013), Heo et al. (2013), Engelhart et al. (2002), Kontush et al. (2001), Li et al. (2012), Quinn et al. (2003), Montilla-López et al. (2002), De Oliveira et al. (2012), Morales et al. (1989), Rebec et al. (2006), Heiser et al. (2010), Sivrioglu et al. (2007), Dadheech et al. (2006), Dakhale et al. (2005), and Arvindakshan et al. (2003)</td>
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<td>Hashim et al. (2011) and Nassiri-asl et al. (2012);</td>
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<td>4</td>
<td>Alzheimer's disease, Parkinson's disease</td>
<td>Elimination of ROS/RNS, Chelating activity, Inhibition of amyloid-β (Aβ) aggregation</td>
<td>Lee et al. (2009), Ono et al. (2004), Ono and Yamada (2012), Gackowski et al. (2008), Sutachan et al. (2012), King et al. (1992) and Rao et al. (2003)</td>
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Table 2. Phenolic compounds with therapeutic potential for the treatment of neurodegenerative diseases.

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<th>Mechanisms involved</th>
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<tbody>
<tr>
<td>1</td>
<td>Alzheimer's, Parkinson's, Huntington's and Schizophrenia</td>
<td>Elimination of ROS, chelating activity; Regulation of antioxidant enzymes; Lipid peroxidation inhibition; Inhibition of amyloid-β (Aβ) aggregation; Attenuation of deficits in motor coordination.</td>
<td>Liu et al. (2013), Sandhir and Mehrotra (2013), Zhu et al. (2013), Huebbe et al. (2010), Ansari et al. (2009), Lavoie et al. (2009), and Chakraborty et al. (2013)</td>
</tr>
<tr>
<td>2</td>
<td>Alzheimer's and Parkinson's</td>
<td>Elimination of ROS; Lipid peroxidation inhibition; Inhibition of amyloid-β (Aβ) aggregation</td>
<td>Wang et al. (2012), Javed et al. (2012), Moshahid et al. (2012), and Islam et al. (2012)</td>
</tr>
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<td>3</td>
<td>Alzheimer's, Parkinson's, Huntington's and Amyotrophic lateral sclerosis</td>
<td>Elimination of ROS, Inhibition of amyloid-β (Aβ); Modulation of cell signaling pathways</td>
<td>Zhang et al. (2013), Lee et al. (2013), He et al. (2012), Levites et al. (2001), Kim et al. (2010b), Avramovich-Tirosh et al. (2007), Ehrnhoefer et al. (2006), Xu et al. (2006), and Koh et al. (2006)</td>
</tr>
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<td>4</td>
<td>Alzheimer's, Parkinson's, and Schizophrenia</td>
<td>Elimination of ROS; Inhibition of amyloid-β (Aβ) aggregation; Inhibition of the aggregation of tau proteins; Lipid peroxidation inhibition.</td>
<td>George et al. (2013), Ejaz Ahmed et al. (2013), Teixeira et al. (2013), Dietrich-Muszalska et al. (2012), and Lim et al. (2013)</td>
</tr>
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<td>Elimination of ROS; Regulation of antioxidant enzymes; Inhibition of amyloid-β (Aβ) aggregation</td>
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Table 2. Contd.

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<td>Elimination of ROS, chelating activity; Regulation of antioxidant enzymes; Inhibition of amyloid-β (Aβ) aggregation</td>
<td>Zhao et al. (2013a, b)</td>
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<td>Heo et al. (2004), Khan et al. (2012), Ma et al. (2013), Zbarsky et al. (2005), and Sonia et al. (2013)</td>
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<td>Elimination of ROS; Inhibition of amyloid-β (Aβ) aggregation</td>
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<td>Barros et al. (2013), Huang et al. (2013a, b), and Fontanilla et al. (2011)</td>
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<td>Elimination of ROS, chelating activity; Regulation of antioxidant enzymes; Inhibition of amyloid-β (Aβ) aggregation</td>
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<td>Mori et al. (2013) and Pi et al. (2012)</td>
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<td>Elimination of ROS; Inhibition of amyloid-β (Aβ) aggregation; Regulation of antioxidant enzymes; Chelating activity, regulating of expression of SIRT1</td>
<td>Khan et al. (2010), Jin et al. (2008b), Feng et al. (2013), Porquet et al. (2013), Ho et al. (2010), Wang et al. (2011), and Kim et al. (2007)</td>
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</table>

by streptozotocin (STZ) (model used to study the Alzheimer's disease) in rats and the results showed that the gallic acid attenuated the cognitive deficits, increased levels of enzymatic antioxidants (GPx, CAT and SOD) and inhibited lipid peroxidation by the reduction of the MDA levels in brain tissue.

Another substance with great therapeutic potential for treatment of neurodegenerative diseases is resveratrol (Figure 7), a phenolic non-flavonoid compound found in grapes and red wine. This antioxidant compound has been investigated in different in vitro and in vivo studies to treatment of Parkinson's disease, Alzheimer's, Huntington's and amyotrophic lateral sclerosis. In a model of Parkinson's disease induced in rats, it was observed that resveratrol (20 mg/kg) in dopaminergic neurons showed neuroprotective effect by regulating glutathione, glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase and decreasing levels of thiobarbituric acid reactive substances (TBARS). Furthermore, it was observed decreased carbonyl protein levels, phospholipase A2 activity and improvement in content of dopamine (Khan et al., 2010). Resveratrol also showed neuroprotective effects in a cellular model of amyotrophic lateral sclerosis and in a model of Alzheimer's disease in mice, being observed in the hippocampus a significant increase of neuronal survival (Kim et al., de 2007).

Confirming the important role of phenolic compounds in complementary therapy and/or
prevention of neurodegenerative diseases, the results of Bournival et al. (2009) have clearly demonstrated that resveratrol and quercetin are compounds that effectively protect neurons against apoptotic cascade induced by oxidative stress.

Most of the works discussed in this article are mainly supported by data obtained in animal models and thus more studies regarding the therapeutic potential for human health are needed to unravel the role of these antioxidant compounds, which are becoming increasingly important as alternative or complementary therapies to neurodegenerative diseases (Figure 4).

CONCLUSION

Based on the literature, there is good evidence that oxidative stress plays an important role in the pathogenesis of neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, Schizophrenia and amyotrophic lateral sclerosis. In this context, natural antioxidant compounds with different chemical structures that neutralize the damaging effects of ROS have been investigated in preventing neurodegenerative diseases. Most of the items discussed in this review demonstrated the efficacy of antioxidant compounds in treatment of neurodegenerative disorders using animal models or in small clinical studies. However, some studies showed contradictory results regarding the therapeutic potential of antioxidants of natural origin. Thus, new studies approaching clinical applications of antioxidants in the treatment or prevention of neurodegenerative diseases are needed.

Conflict of interest

The author(s) declare(s) that they have no conflicts of interest to disclose.

REFERENCES


Short Communication

Synthesis of some new [1,3,4]oxadiazine-[6,5-b]indole derivatives and their biological activity

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Novel 2-[(benzalamino-4-hydroxybenzyl) [1,3,4]-oxadiazino[6,5-b]]indole derivatives (V1-21) were synthesized via a simple condensation reaction between 2-Amino-4-[(1,3,4]-oxadiazino-[6,5-b]indole-3-yl)-phenol (IV) and challenging aromatic aldehydes. The compound IV was synthesized by an acid catalyzed cyclization of 3-amino-4-hydroxy-benzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (III). Thus produced final compounds (V1-21) were purified using column chromatography and then characterized by various spectroscopic techniques. Finally, the title compounds were screened for antimicrobial activity and shown promising results when compared with standard compounds.

Key words: [1,3,4]Oxadiazine-[5,6-b]indole, aromatic aldehydes, antimicrobial activity.

INTRODUCTION

Extensive studies proved that indole derivatives exhibit varied biological and pharmacological properties including; Antimicrobial (Pandeya et al., 1999), antiviral (Jarrahpour et al., 2007), anticancer (Vine et al., 2009), analgesic (Pandeya et al., 2005), central nervous system (CNS) activities (Verma et al., 2004). These precious properties inspired me to develop the new [1,3,4]oxadiazino-[5,6-b]- indole derivatives (V) as potent antimicrobial agents (Scheme 1). Indoe-2,3-diones (I) were prepared and treated with 3-amino-4-hydroxybenzoicacidhydrazide (II) in ethanol to produce 3-Amino-4-hydroxy-benzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (III). These compounds were cyclized using concentrated sulfuric acid to obtain 2-Amino-4-[(1,3,4)-oxadiazino[6,5-b]indole-3-yl)-phenol (IV). Finally, these compounds were refluxed with aromatic aldehyde in ethanol and few drops of acetic acid to produce the final compounds as shown in Scheme 1. The compounds were characterized by their physical, analytical and spectral data [infra-red (IR) and nuclear magnetic resonance (NMR)]. The list of final compounds with their melting point, synthetic yield and retention factor (Rf) values are given in the Table 1. The elemental analysis results are given in the Table 2. The antimicrobial effectiveness of title compounds is represented in Table 3.

METHODOLOGY

Experimental

The melting points (in °C) were recorded in open capillaries using

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Toshniwal melting point apparatus and are uncorrected. The IR spectra were recorded on Perkin-Elmer Infracord-283 spectrophotometer. NMR spectra were recorded on OMEGA-500mHz spectrophotometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded by the direct inlet method on FINNINIGAN MAT-90 in the El mode. Isatins (I) and 3-amino-4-hydroxybenzoic acid hydrazide (II) were synthesized by simple cyclization methods (Gassman et al., 1977; Hewawasam et al., 1994; Chiyanzu et al., 2003).

3-Amino-4-hydroxy-benzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (III)

An appropriate isatin (I, 0.01 mol) was heated under reflux, in ethanol (50 ml) with 3-amino-4-hydroxybenzoic acid hydrazide (II, 0.01 ml) for 1.5 h. The product so separated was filtered and
Table 1. Physical Data of New [1,3,4]oxadiazino[5,6-b]indole derivatives.

<table>
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<tr>
<th>Compound</th>
<th>Substituents</th>
<th>Molecular formula</th>
<th>MP (°C)</th>
<th>Rf Value</th>
<th>Yield (%)</th>
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<td>V(1)</td>
<td>F H H H</td>
<td>C₂₂H₁₃N₄O₂F</td>
<td>268</td>
<td>0.659</td>
<td>88</td>
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<tr>
<td>V(2)</td>
<td>F H Cl H</td>
<td>C₂₂H₁₂N₂O₂ClF</td>
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<td>0.557</td>
<td>86</td>
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<tr>
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<td>85</td>
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<tr>
<td>V(4)</td>
<td>F H OCH₃ H</td>
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<td>233</td>
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<td>89</td>
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<tr>
<td>V(5)</td>
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<td>0.670</td>
<td>90</td>
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<tr>
<td>V(6)</td>
<td>F H N(CH₂)₂ H</td>
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<td>&gt;300</td>
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<td>86</td>
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<tr>
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<td>F H OH OCH₃</td>
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<td>Cl H H H</td>
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<td>0.721</td>
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<td>V(10)</td>
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<tr>
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<td>&gt;300</td>
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<td>0.742</td>
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<td>C₂₅H₁₈N₂O₂Cl</td>
<td>256</td>
<td>0.889</td>
<td>81</td>
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Table 2. Analytical data of New [1,3,4]oxadiazino[5,6-b]indole derivatives.

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Calculated</th>
<th>Found</th>
</tr>
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<tr>
<td></td>
<td>C% H% N% O% Cl% F% C% H% N% O% Cl% F%</td>
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<td>V(1)</td>
<td>68.75 3.41 14.58 8.33 - 4.94 69.01 3.23 14.65 8.78 - 4.78</td>
<td></td>
</tr>
<tr>
<td>V(2)</td>
<td>63.09 2.89 13.38 7.64 8.47 - 4.54 63.21 2.98 13.78 7.90 8.89 4.98</td>
<td></td>
</tr>
<tr>
<td>V(3)</td>
<td>66.00 3.27 13.99 11.99 - 4.75 69.90 3.78 13.89 11.09 - 4.56</td>
<td></td>
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<tr>
<td>V(4)</td>
<td>66.66 3.65 13.58 11.58 - 4.58 67 3.98 13.09 11.56 - 4.43</td>
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<tr>
<td>V(5)</td>
<td>64.86 3.86 12.61 14.40 - 4.27 64.36 3.90 12.78 14.36 - 4.23</td>
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<tr>
<td>V(7)</td>
<td>64.19 3.51 13.02 14.87 - 4.41 64.57 3.87 13.90 14.97 - 4.25</td>
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<td>65.92 3.27 13.98 7.98 8.85 - 65.87 3.56 13.78 7.45 8.66 -</td>
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<td>V(9)</td>
<td>60.71 2.78 12.87 7.35 16.29 - 61.07 2.90 12.13 7.24 16.87 -</td>
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<td>V(10)</td>
<td>63.39 3.14 13.44 11.52 8.51 - 64.30 3.23 13.45 11192 8.66 -</td>
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<td>V(11)</td>
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<td></td>
</tr>
<tr>
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<td>62.55 3.72 12.16 13.89 7.69 - 63.01 3.89 12.17 13999 7.25 -</td>
<td></td>
</tr>
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<td>V(13)</td>
<td>69.94 4.09 15.78 7.21 7.99 - 70.00 4.21 15.34 7.46 7.76 -</td>
<td></td>
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<tr>
<td>V(14)</td>
<td>61.82 3.38 12.54 14.32 7.93 - 61.89 3.46 12.09 14.89 7.87 -</td>
<td></td>
</tr>
<tr>
<td>V(15)</td>
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</tr>
<tr>
<td>V(16)</td>
<td>66.59 3.64 13.51 7.71 8.55 - 66.56 3.89 13.56 7.89 8.23 -</td>
<td></td>
</tr>
<tr>
<td>V(17)</td>
<td>69.99 4.07 14.13 12.11 - - 69.90 4.01 14.46 12.67 -</td>
<td></td>
</tr>
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<td>V(18)</td>
<td>70.23 4.42 13.65 11.69 - - 70.48 4.56 13.98 11.86 -</td>
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</tr>
<tr>
<td>V(19)</td>
<td>68.17 4.58 12.72 14.53 - - 68.99 4.78 12.90 14.90 -</td>
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<tr>
<td>V(20)</td>
<td>70.91 5.00 16.54 7.56 - - 71.09 5.05 16.34 7.54 -</td>
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</tr>
<tr>
<td>V(21)</td>
<td>67.60 4.25 13.14 15.01 - - 67.98 4.56 13.14 15.89 -</td>
<td></td>
</tr>
</tbody>
</table>

C = carbon, H = hydrogen, N = nitrogen, O = oxygen, Cl = chlorine, F = fluorine.
Table 3. Data on antimicrobial Activity of New [1,3,4]oxadiazino-[5,6-b]indole derivatives.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>B. Subtilis</th>
<th>S. Aureus</th>
<th>E. coli</th>
<th>P. vulgaris</th>
<th>A. niger</th>
<th>C. verticulata</th>
<th>F. oxysporum</th>
<th>A. flavus</th>
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<td>Ampicillin (10 µg/cup)</td>
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<td>-</td>
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<td>22</td>
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<tr>
<td>Clotrimazole (10 µg/cup)</td>
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*Concentration of test compound: 100 µg/cup.

purified by recrystallization from suitable solvents. The physical constants were compared with the literature values.

2-Amino-4-([1,3,4]oxadiazino[6,5-b]indole-3-yl)-phenol (IV)

An appropriate 3-amino-4-hydroxy-benzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (III, 0.01 mol) was dissolved in 10 ml of concentrated sulphuric acid. The reaction mixture was kept aside for 2 h, poured on to crushed ice and neutralized with sodium bicarbonate solution. The product so separated was filtered and recrystallized from suitable solvents.

2-[(benzalaminio-4-hydroxybenzyl)oxadiazino(6,5-b)]indole (V)

Each of the 2-amino-4-([1,3,4]oxadiazino[6,5-b]indole-3-yl)-phenol (IV, 0.01 mol) was heated with an aromatic aldehyde (benzaldehyde, p-chloro benzaldehyde, salicylaldehyde, anisaldehyde, veratraldehyde, p-dimethylamino benzaldehyde and vanilaldehyde) in ethanol (20 ml) and few drops of acetic acid, heated under reflux on water bath for 3 h. The solvent was removed to the possible extent by distillation under reduced pressure. The product thus obtained was filtered, washed with water and purified by recrystallization from appropriate solvent (Table 1). For example, 2-amino-4-(8-fluoro-[1,3,4]oxadiazino[6,5-b]indole-3-yl)phenol (IV, R = F) was condensed with benzaldehyde to make a particular product. This on purification by
recrystallization from methanol and DMF (1:1) resulted in yellow color, solid, m.p. 286°C. It was characterized as 2-(benzylideneamino)-4-(8-fluoro-[1,3,4]oxadiazino-[6,5-b]indol-3-yl)phenol (V (1)). Its IR spectrum (in KBr) showed characteristic absorption bands (in cm\(^{-1}\)) at 1,610 (C=N), 1100 (C-O-C). PMR spectrum showed characteristic signals (in \(\delta\) ppm) at 12.5 (s, 1H, -OH), 7.1 to 8.9 (m, 11H, Ar-H).

**Antimicrobial activity**

The antibacterial activity of the test compounds was assayed against *Bacillus subtilis*, *Staphylococcus aureus* (gram-positive) and *Escherichia coli* and *Proteus vulgaris* (gram-negative) by CUP-plate method (Indian pharmacopoeia, 1996). The antifungal activity (Table 2) of test compounds was determined against *Aspergillus niger*, *Conradina verticillata*, *Fusarium oxysporum* and *Aspergillus flavus* by the cup-plate method (British Pharmacopoeia, 1953).

**RESULTS AND DISCUSSION**

The title compounds were characterized by their physical, analytical and spectral data. The details of the compounds have been given in the experimental section. The antibacterial details of 2-[(benzalamino)-4-hydroxybenzyl] (1,3,4)-oxadiazino(6,5-b)indoles (V) indicate that these compounds exhibited a minimal antibacterial activity, interestingly, against all the four strains of bacterial and near to the same extent. The compounds exhibit antifungal activity against all the four strains fungi employed but of course with a degree of variation. These compounds were found to be somewhat more effective against *A. niger*, *Conradina verticillata*.

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**Conflict of interest**

The author(s) declare(s) that they have no conflicts of interest to disclose.

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- African Journal of Pharmacy and Pharmacology
- Journal of Dentistry and Oral Hygiene
- International Journal of Nursing and Midwifery
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- Journal of Pharmacognosy and Phytotherapy
- Journal of Toxicology and Environmental Health Sciences