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(RESEARCH ARTICLE)



Cytoprotective action of *Griffonia simplicifolia* (DC.) Baill. against the oxidative stress caused by hydrogen peroxide (H₂O₂) on neurons and astrocytes

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Abstract

Cerebral malaria and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis are characterized by a significant increase in oxidative stress that can lead to cellular damage to neurons and astrocytes. Therefore, the search for molecules capable of modulating oxidative stress in these diseases has recently gained interest, particularly for non-traditional antioxidants that can be obtained from plants. In this work, the aqueous leaf extract of *Griffonia simplicifolia* was used to evaluate its ability to protect both neurons and astrocytes against oxidative stress induced by hydrogen peroxide. The analysis of the chemical antioxidant activity showed that *Griffonia simplicifolia* has a good antioxidant activity with an IC₅₀ equal to 85.11 µg/mL. Toxicity tests showed that the aqueous extract of *Griffonia simplicifolia* did not affect the cellular viability of neurons and astrocytes (IC₅₀ > 800 µg/mL). In addition, at a concentration of 20 µg/mL, the aqueous extract of *Griffonia simplicifolia* protected both neurons and astrocytes against H₂O₂-induced oxidative stress. Our results therefore suggest that the aqueous extract of *Griffonia simplicifolia* contains antioxidant molecules that may have therapeutic potential.

Keywords: Antioxidants; Cerebral malaria; Cytoprotective; *Griffonia simplicifolia*; Neurodegenerative diseases

1. Introduction

Cerebral malaria and neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and Huntington's disease are characterized by loss of neuronal function and memory and cognitive impairment [1]. Oxidative stress, including lipid peroxidation, free radical formation, protein oxidation and DNA oxidation, in the central nervous system (CNS) can lead to cell death and contributes to the pathogenesis of various neurodegenerative disorders [2, 3].

Reactive oxygen species (ROS) regulate cellular signaling pathways related to the cell cycle, proliferation, and apoptosis [4] and are associated with altered cellular oxidation and impaired cellular function. ROS include the superoxide radical anion (O₂⁻), hydroxyl radical (OH[·]), singlet oxygen (¹O₂), and hydrogen peroxide (H₂O₂) [5].

H₂O₂ is one of ROS that, in its electrically neutral form, diffuses through cellular membranes [6]. Exogenous H₂O₂ is considered to be a mediator of apoptosis that induces oxidative stress in neuronal cells. Hydroxyl radicals derived from H₂O₂ can lead to cytotoxicity in neuronal cells and downregulate antioxidant enzyme expression, inducing caspase-3 activation. Thus, H₂O₂ has been widely used to damage PC-12 cells derived from rat pheochromocytoma [6].

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Griffonia simplicifolia (syn. *Bandeiraea simplicifolia* Benth.) is a climbing leguminous plant native to West Africa notably in Ghana, Liberia, Togo and Côte d'Ivoire. It belongs to the Fabaceae family. *Griffonia simplicifolia* is used in herbal medicine mainly for diseases requiring an increase in serotonin concentration or its regulation [7]. Used in various forms (tinctures, tablets, decoctions, extracts, etc.) it is an effective natural alternative to SSRI and synthetic antidepressants for the treatment of mood disorders, anxiety and depression [8] and also used as secondary treatment for sleep disorders and appetite [9, 10]. In order to modulate oxidative stress in these diseases we have evaluated the ability of *G. simplicifolia* to protect neurons and astrocytes against oxidative stress induced by hydrogen peroxide.

2. Material and methods

2.1. Collection of plants material

Griffonia simplicifolia was collected in Department of Agboville (South-Eastern of the Ivory Coast). The plant material was identified by Floristic Center of Félix Houphouët-Boigny University. A voucher herbarium specimen is deposited at the Floristic Center.

2.2. Reagent

Sodium bicarbonate, Minimal essential medium (MEM), Heat-inactivated Foetal Bovine Serum (FBS), Trypsin, Gentamycin, Penicillin-Streptomycin solution (Penstrep) were used for PDA (Progenitor Derived Astrocytes) and SH-SY5Y-Neuron culture.

2.3. Preparation of crude extracts

After the harvest, the leaves of the plants were in the shelter of the sun for two weeks at ambient temperature before being reduced to fine powder by crushing using a mechanical crusher. According to the protocol of extraction of hundred grams (100 g) of powders was solubilized in one liter of distilled water by crushing in Blender during 10 to 15 minutes [11]. The homogenate obtained is initially dried in a fabric square, then filtered successively twice on absorbent cotton and once on paper Whatman 3 mm. The filtrate obtained was dried at 50 °C.

2.4. Antioxidant activity (DPPH) assay

The evaluation of the antioxidant potential of the extracts was done according to the method of Blois [12]. In the presence of antiradical compounds, the purple-colored DPPH (2, 2-diphenylpicrylhydrazyl) radical is reduced and then changes color turning yellow. DPPH is solubilized in absolute ethanol to obtain a 0.03 mg/mL solution. Then, different concentrations of the extracts of 1 to 0.001 mg/mL were prepared by double dilution in absolute ethanol. In test tubes containing 2.5 mL of an extract at a given concentration, 1 mL of DPPH at 0.03 mg/mL was added. The tubes were subsequently incubated for 30 min in the dark. The absorbance of each tube was determined using spectrophotometer at 517 nm against a blank containing no extract. The positive control was represented by a vitamin C solution (standard antioxidant).

The absorbance measured at 517 nm were used to calculate the percent inhibition of the DPPH radical, which is proportional to the antiradical power of the sample. The percent inhibition of DPPH representing the antioxidant activity was calculated according to the formula given below and the concentrations necessary to trap 50% of the DPPH (IC₅₀) were determined with the Graph pad prism software.

The antioxidant activity of the extracts was classified according to the following IC₅₀ values in µg/mL [12]:

IC₅₀ < 50 µg/mL: Very good antioxidant activity

50 µg/mL < IC₅₀ < 100 µg/mL: Good antioxidant activity

100 µg/mL < IC₅₀ < 250 µg/mL: Moderate antioxidant activity

250 µg/mL < IC₅₀ < 500 µg/mL: Low antioxidant activity

500 µg/mL < IC₅₀: No antioxidant activity

2.5. Neural Stem Cell (NSC) culture

2.5.1. Differentiation of NSC stem cells to astrocytes

NSCs were derived and maintained in continuous culture for at least one week in NSC media. For differentiation, the culture media of the NSCs were replaced by the MEM (Minimum Essential Media) media containing 10% FBS. Maintenance of the culture by half media change (every alternate day) was done for twenty-one days in order to obtain mature astrocytes [13].

2.5.2. Differentiation of SH-SY5Y cells to neurons

SH-SY5Y cells were maintained in continuous culture for at least one week. For differentiation, the old SH-SY5Y cell culture media was removed and replaced with a new MEM media containing 10 μ M retinoic acid. Maintenance of the culture by half media change every alternate day (media containing retinoic acid) was done for seven days to obtain neurons [14].

2.5.3. Co-culture of neurons and astrocytes

After 21 days in culture, the astrocytes were washed with PBS and incubated in a trypsin-EDTA solution (0.05 and 0.02%, respectively) for 5 minutes at 37 °C. After incubation, the cells were centrifuged at 1000 rpm for 5 min. The cell pellet (astrocytes) was resuspended in the MEM containing 10% FBS and seeded in flasks. Next day, SHSY-5Y neurons were seeded over the astrocytes (in ratio A: N:: 2:1).

2.6. Cytotoxicity of plant extract

2.6.1. Treatments

Human PDAs or SHSY-5Y neurons were cultured in 96-well culture plates at a density of 15,000 cells/well, and incubated at 37 °C for 24 h. When Cells were approximately 80% confluent, media was removed and replaced by the different concentrations of plant extracts: 800 - 400 - 200 - 100 - 50 - 25 - 12.5 - 6.25 - 3.125 μ g/mL (100 μ L per well). Plate was incubated for 48 h at 37 °C.

2.6.2. MTT Assay for assessing Cell Viability

After appropriate time intervals, the media was removed and replaced by 100 μ L growth medium with 0.5 mg/mL MTT, and the plates were incubated for an additional 3 h at 37 °C. Subsequently, the supernatant was removed and replaced by 100 μ L of solubilization solution (50% DMF and 20% SDS) to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using a 96-well multi scanner autoreader. The results were presented as a percentage of viable cells as compared to the control.

2.7. Protective effect of aqueous leaf extract of *G. simplicifolia* against oxidative stress induced by H₂O₂

2.7.1. Toxicity of H₂O₂

Cellular toxicities of H₂O₂ were determined in human PDAs and SHSY-5Y neurons using the MTT assay. Cells were cultured in 96-well culture plates at a density of 15,000 cells/well, and maintained in 5% CO₂ at 37° C for 24 h. After the incubation with indicated concentrations of H₂O₂, cell viability was determined by MTT assay. Control groups consisted of cells incubated with media only. After appropriate time intervals, the media was removed and replaced by 100 μ L growth medium with 0.5 mg/mL MTT, and the plates were incubated for an additional 3 h at 37° C. Subsequently, the supernatant was removed and replaced by 100 μ L of solubilization solution (50% DMF and 20% SDS) to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using a 96-well multiscanner autoreader. The results were presented as a percentage of viable cells as compared to the control.

2.7.2. Protective effect against H₂O₂ injuries

Cells were seeded into 96-well culture plates at a density of 15,000 cells/well. Twenty-four hours after seeding, cells were then pretreated for 1 h with our extract diluted in serum-free media at concentrations of 20 μ g/mL. The treated cells were then challenged with 500 μ M H₂O₂ for 3 h. Then, H₂O₂ was removed and replaced by 100 μ L growth medium with 0.5 mg/mL of MTT was added to all wells and allowed to incubate in the dark at 37° C for 3 h. The amount of MTT formazan product was determined by measuring absorbance using a microplate reader at 570 nm.

3. Results and discussion

3.1. Antioxidant activity

The IC_{50} value of inhibition of DPPH by *G. simplicifolia* is 85.11 $\mu\text{g/mL}$ and vitamin C is 13 $\mu\text{g/mL}$. According to the classification of Blois [12], aqueous extract of *G. simplicifolia* have good antioxidant activity ($50 \mu\text{g/mL} < IC_{50} < 100 \mu\text{g/mL}$). The antioxidant activity of these extract is related to the presence of secondary metabolites. Indeed, studies have shown that secondary metabolites such as saponins and triterpenes [15], all derived from plants have good antioxidant activities [16]. The antioxidant activity of these extract could be explained more precisely by the presence of saponins. Because the hydroxyl group (OH) directly linked to the benzene ring of these natural compounds, thus allow them to easily give electrons to the electron-deficient free radicals and to chelate the ions of the transition metals capable of catalyzing lipid peroxidation in the body to reduce their threats in biological systems [17, 18].

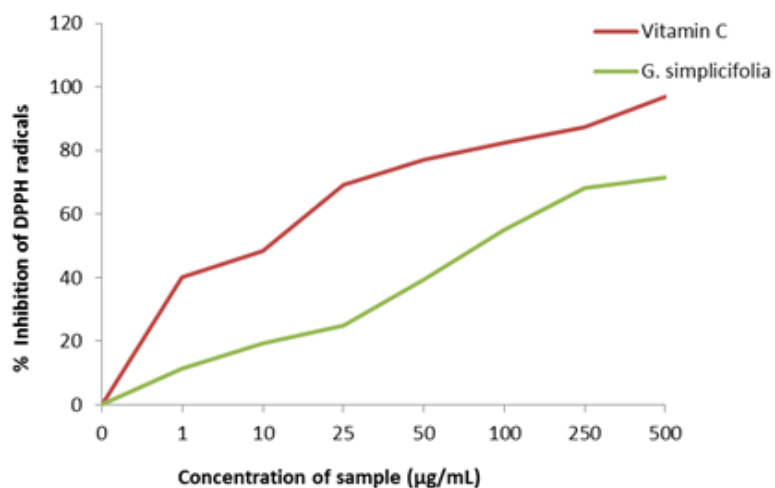


Figure 1 Percent of inhibition of DPPH by vitamin C and *G. simplicifolia*

3.2. Cytotoxicity of plant extract

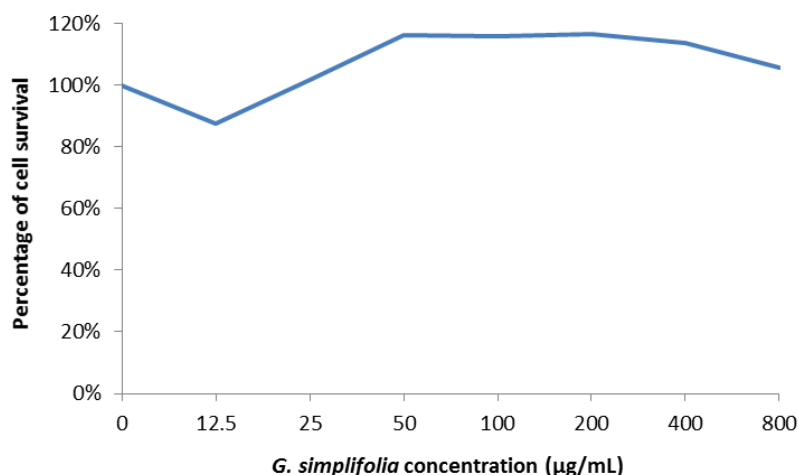


Figure 2 Cytotoxicity of *G. simplicifolia*

The study of the cytotoxicity of this plant used in the traditional treatment of malaria [19] has revealed that in addition to their antimalarial activity these plants are non-toxic to human brain cells. The property of these plants in water-soluble tannins may explain their low toxicity [20-21].

3.3. Protective effect

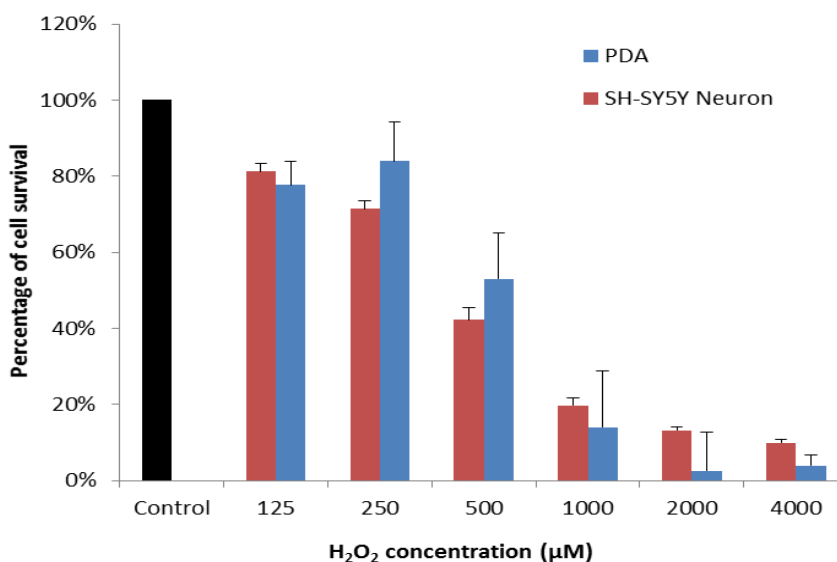


Figure 3 Cytotoxicity of H₂O₂ on mammalian cells

G. simplicifolia extract exhibited significant neuroprotective effects by reducing H₂O₂-induced cell death to 16 ± 5%. Up to now, it has been clearly shown that one common mechanism that mediates cellular longevity and healthful aging is protection against oxidative stress [22]. Notably, an excessively high level of reactive oxygen species (ROS) with normal levels of endogenous antioxidant enzymes is the basis for oxidative stress in the brain, which causes apoptosis and cell damage. In view of this, the use of exogenous antioxidants has been proposed as a method for managing ROS sequelae including damage to neuronal cells [23]. The present study demonstrates that *G. simplicifolia* protects human brain cells against H₂O₂-induced oxidative stress.

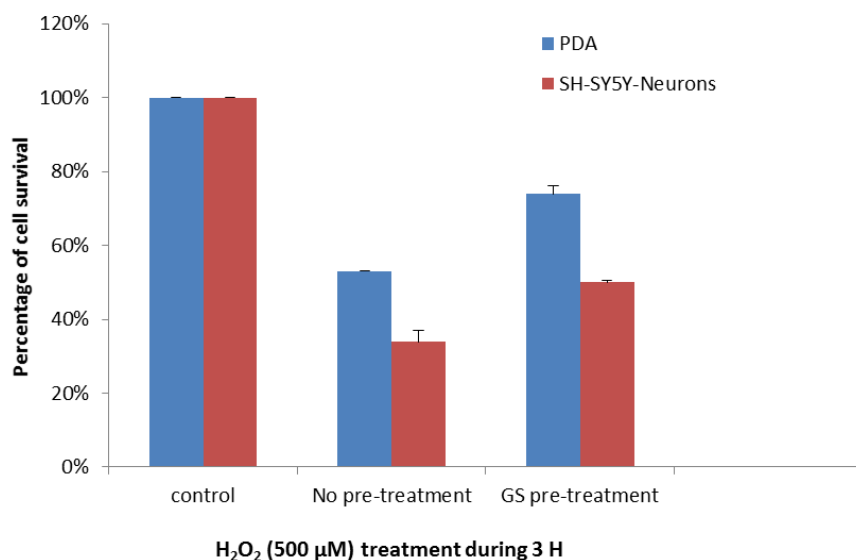


Figure 4 Protective effect of aqueous extract of *G. simplicifolia* against H₂O₂ toxicity

4. Conclusion

We have been able to show that *G. simplicifolia* extract protects neurons and astrocytes against oxidative stress. This plant could be used in the manufacture of a drug against neurodegenerative diseases. In the rest of the study, we plan to isolate and characterize the molecules responsible for this antioxidant effect.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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