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Inhibitory effects Induced by Vicia faba, Uncaria rhyncophylla, and Glycyrrhiza glabra water extracts on oxidative stress biomarkers and dopamine turnover in HypoE22 cells and isolated rat striatum challenged with 6-hydroxydopamine

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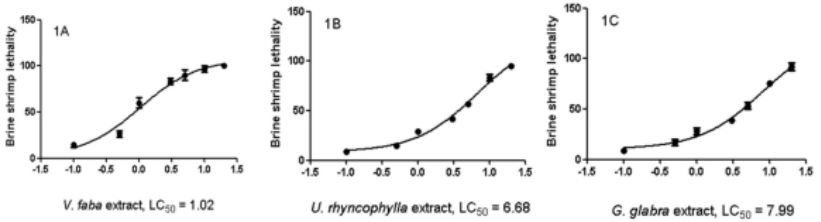
Graphical Abstract

Biocompatibility

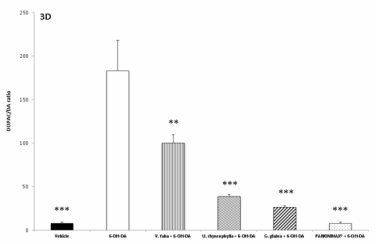


Phytochemical analysis

Phenolic Compound	*V. faba* (mg/g dry Extract)	*U. rhyncophylla* (mg/g Dry Extract)	*G. glabra* (mg/g Dry Extract)
Gallic acid	3.91 ± 0.35	30.80 ± 2.77	16.96 ± 0.68
Catechin	4.92 ± 0.59	6.11 ± 0.73	12.71 ± 0.99
Epicatechin	n.d.	n.d.	1.06 ± 0.05
Resveratrol	2.46 ± 0.25	25.25 ± 2.53	63.23 ± 7.14

n.d.: not determined.

Protective effects



Abstract: Parkinson's disease (PD) is the most common and progressive neurodegenerative and oxidative stress-related disorder, characterized by a dramatic loss of dopamine (DA) neurons in the nigrostriatal tissue. The first-line drug for PD treatment is represented by L-dopa, although clinical and preclinical studies pointed out the potential efficacy of medicinal plant- and food-derived antioxidants as brain protective agents. In this regard, the potential application of *Vicia faba*, *Uncaria rhyncophylla*, and *Glycyrrhiza glabra* extracts is of noteworthy interest, despite a lack of information in the scientific literature as regards their effect on striatal DA level.

The protective effects of *V. faba*, *U. rhyncophylla*, and *G. glabra* water extracts were investigated on HypoE22 cells and isolated rat striatum specimens challenged with 6-hydroxydopamine (6-OH-DA). The extract effects against lactate dehydrogenase (LDH), nitrites, and 8-*iso*-prostaglandin(PG)F2α were evaluated using either single-extract treatments or a treatment with a pharmacological association. Additionally, the turnover of DA was measured.

The pharmacological association of the extracts was the most effective in contrasting the upregulated LDH and nitrite levels and in reducing striatal DA turnover.

The present findings corroborate the rationale for the traditional use of *V. faba*, G. glabra, and *U. rhyncophylla* extracts, supporting their pharmacological association in order to improve their protective effects.

Keywords: Parkinson'disease; *Vicia faba; Uncaria rhyncophylla; Glycyrrhiza glabra*; dopamine.

Introduction

Oxidative/nitrosative stress plays a master role in age-related cell degeneration, particularly in the brain, where the increased burden of peroxide-modified unsaturated fatty acids is coupled with the inhibition of antioxidant systems. The adult brain displays a high level of unsaturated fatty acids which are highly sensitive to peroxidation induced by mitochondrial-derived reactive oxygen/nitrogen species (ROS/RNS). The intrinsic modest antioxidant defense coupled with neurotransmitter autoxidation is also causally related to the brain susceptibility to oxidative/nitrosative stress. Parkinson's disease (PD) is the most common and progressive neurodegenerative and oxidative stress-related disorder, characterized by a dramatic loss of dopamine (DA) neurons in the nigrostriatal tissue, which in turn causes impairment in voluntary movement control. Actually, more than 10 million people worldwide are affected by PD, with the incidence increasing progressively with age, despite there being 4% of PD patients under 50 years of age, particularly in the male population. The first-line drug is represented by l-dopa that is able to contrast, albeit partially, the loss of DA in the nigrostriatal tissue. Nevertheless, long-term benefits related to l-dopa administration are inconsistent. For this purpose, l-dopa is co-administered with amino acid decarboxylase inhibitors, including carbidopa and benserazide, in order to reduce the peripheral conversion of l-dopa to DA and the consequent peripheral (nausea, emesis) and central (dyskinesia, on–off phenomenon) side effects. In PD, the activity of monoamino oxidase B (MAO-B) is also increased, thus leading to both DA depletion and increased mitochondrial-derived ROS production. Clinical and preclinical studies pointed out the potential efficacy of medicinal plant- and food-derived antioxidants as protective agents in the brain of both young and aged rats [9,10]. Additionally, herbal extracts and isolated phytocompounds able to counteract the burden of oxidative stress and inflammation in the brain revealed efficacy in restoring tissue neurotransmitter levels in both in vitro and in vivo experimental models of neurodegeneration, possibly through multiple concomitant mechanisms . Furthermore, considering the multifactorial metabolic aspects of PD, the pharmacological association of medicinal plant-derived extracts could ameliorate their efficacy. Several herbal extracts were found to be effective in experimental models of PD, particularly for their capability to contrast the increased burden of inflammation/oxidative stress and improve DA signaling. In this regard, the potential application of Vicia faba, Uncaria rhyncophylla, and Glycyrrhiza glabra extracts as adjuvant agents in the management of clinical symptoms related to PD is of noteworthy interest. V. faba represents the elective natural source of l-dopa, which was isolated from beans by Torquato Torquati in 1910–1911, and whose structure was described by Markus Guggenheim in 1913. Case report studies also indicated the efficacy of V. faba beans in improving the motor function in PD patients, through the prolonging of "on" periods, following the ingestion of a large bean meal. A recent double-blind clinical trial also pointed out the efficacy of a polyphenol-rich G. glabra extract, administered as adjuvant therapy, in improving the motor function in PD patients. U. rhyncophylla, besides being traditionally used in Northern Asia as an anti-Parkinson remedy, was shown to be effective as a neuroprotective agent in PC12 cells challenged with 6-hydroxydopamine (6-OH-DA), a well-recognized pro-inflammatory and pro-degenerative stimulus employed in in vitro and in vivo models of PD. Nevertheless, there is still a lack in the scientific literature as regards the putative role of these medicinal plants in modulating DA levels in the striatum. With the aim to further elucidate the mechanism of action of these herbs in PD treatment, in the present work, the protective effects of V. faba, U. rhyncophylla, and G. glabra water extracts were investigated in an experimental model of neurotoxicity consisting of neuronal HypoE22 cells and isolated rat striatum specimens challenged with 6-OH-DA. The protective effects of the extracts were evaluated by analyzing selected biomarkers of cytotoxicity and nitrosative and oxidative stress, namely, lactate dehydrogenase (LDH), nitrites, and 8-iso-prostaglandin(PG)F2α, respectively, using both single-extract treatments and a pharmacological association (PARKININAX®). In the same condition, DA turnover was measured as well and expressed as the ratio between dihydroxyphenilacetic acid (DOPAC) and DA levels. Finally, in order to provide a better interpretation of the observed pharmacological effects, a fingerprint analysis was carried out on selected phenolic compounds, namely, gallic acid, catechin, epicatechin, and resveratrol, which are known to exert protective effects at both central and peripheral level via multiple mechanisms. In this regard, gallic acid, besides having anti-radical effects, especially at low to moderate concentrations, was recently described to exert protective effects in an experimental PD model in vitro; in addition, catechin intake was related to a lower risk of PD, possibly through a regulatory effect on neuronal viability and synaptic plasticity. Finally, resveratrol displayed an intriguing efficacy against PD both in vitro and in vivo, being also able to synergize with l-dopa

.

Results and discussion

The HPLC–fluorimetric analysis was focused on selected phenolic compounds, namely, gallic acid, catechin, epicatechin, and resveratrol. The results indicated that the U. rhyncophylla water extract was the richest in gallic acid, whereas the G. glabra water extract displayed the best qualitative profile alongside with higher levels of catechin, epicatechin, and resveratrol. Conversely, the V. faba water extract displayed the poorest qualitative and quantitative profiles, as regards the selected phenolic compounds. The V. faba extract was also assayed via HPLC–EC for quantifying its level of l-dopa (33.71 ± 4.21 µg/g dry extract). The results of the HPLC-fluorimetric analysis were consistent with the colorimetric evaluations of total phenols (expressed as mg of gallic acid per g of dry extract) previously described in the literature, although a punctual description of the U. rhyncophylla phenolic profile is still lacking. The concentration of l-dopa found in the V. faba extract was consistent with the content of this compound in green plant pods.

Phenolic Compound	*V. faba* (mg/g dry Extract)	*U. rhyncophylla* (mg/g Dry Extract)	*G. glabra* (mg/g Dry Extract)
Gallic acid	3.91 ± 0.35	30.80 ± 2.77	16.96 ± 0.68
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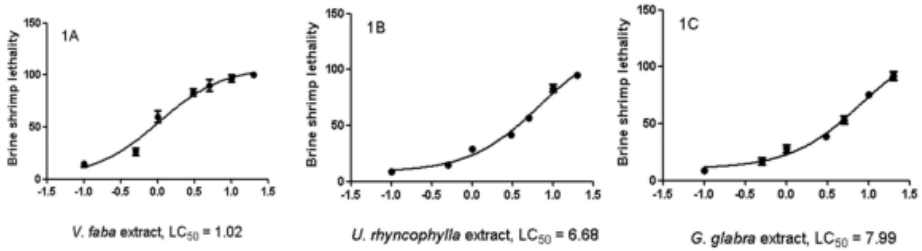
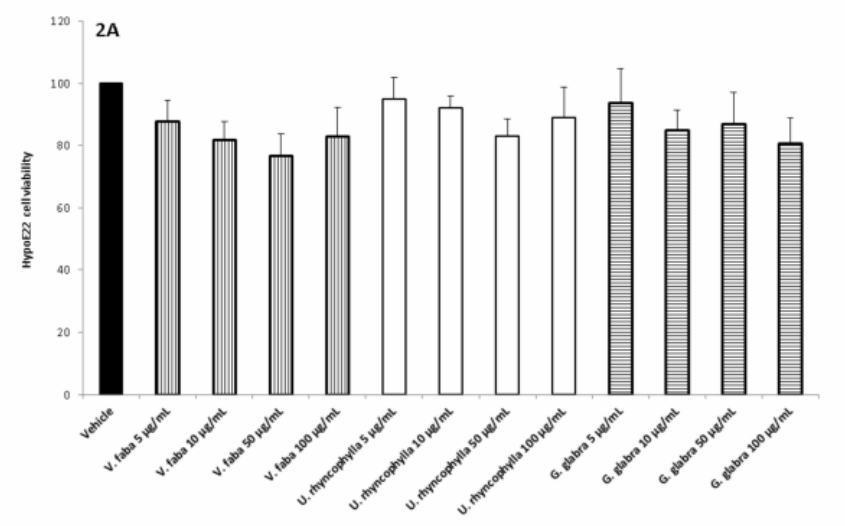
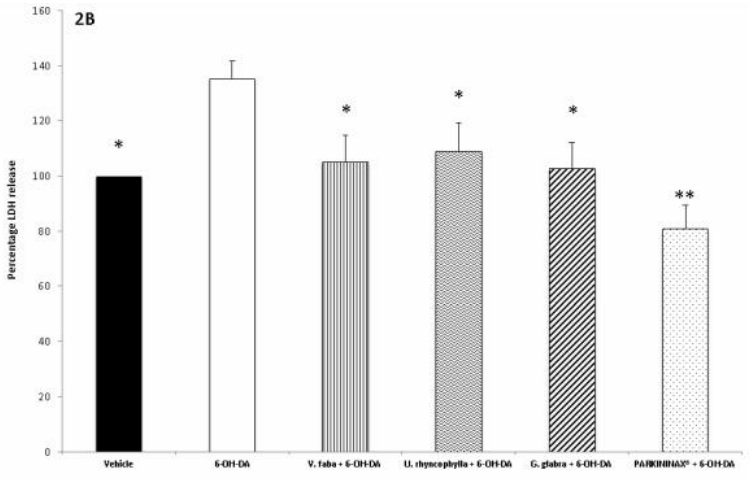
n.d.: not determined.

Results and discussion

The three extracts were tested for their neuroprotective role after evaluating their biocompatibility (0.1–20 mg/mL) through a lethality test in the brine shrimp A. salina Leach. Since the results indicated LC50 values >1 mg/mL (Figure 1), a concentration range at least 10-fold lower was selected for the in vitro viability (MTT) assay on the HypoE22 cell line. Particularly, the MTT test revealed that all the three extracts (5–100 µg/mL) either alone or in pharmacological association (Formula) did not significantly modify HypoE22 cell viability, with a percentage viability in the range of 70–100% (Figure 2A).

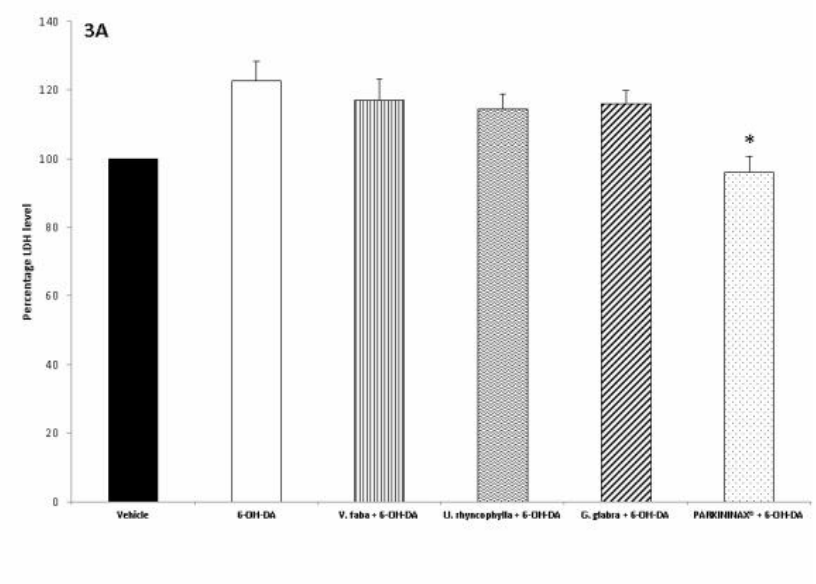


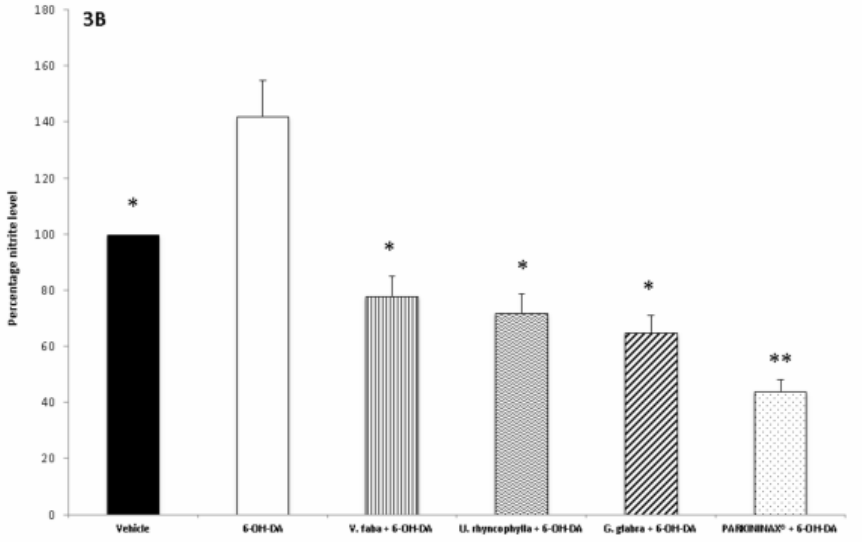




Results and discussion

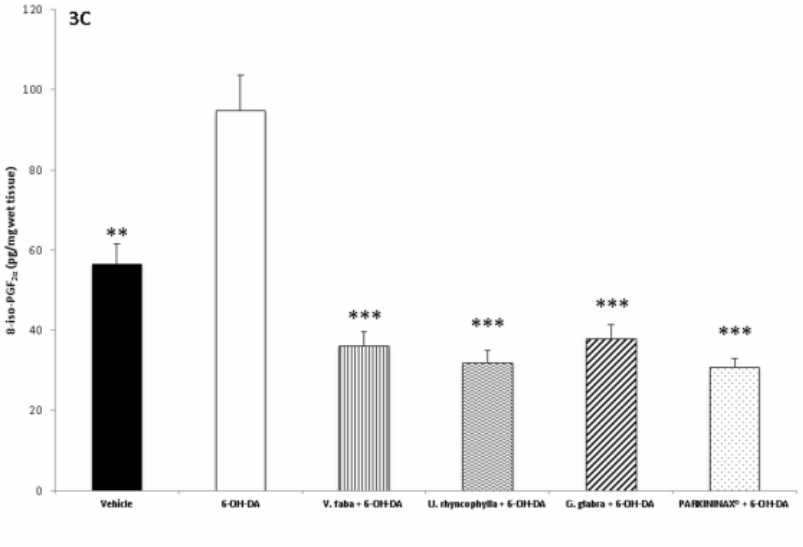
Considering the results of the MTT test and the aforementioned ratio of the single ingredients in the formula (PARKININAX®), the following extract concentrations were selected for the subsequent in vitro and ex vivo assays in HypoE22 cells and isolated rat striatum specimens challenged with 6-OH-DA: V. faba, 85 µg/mL; G. glabra, 10 µg/mL; U. rhyncophylla, 50 µg/mL. Being PARKININAX® a classical pharmacological association, the resulting concentration was the algebric sum (100 µg/mL) of the concentrations of the selected extracts used in single treatments. Within the employed in vitro and ex vivo experimental paradigms, selected biomarkers of citotoxicity and nitrosative and oxidative stress, namely, LDH, nitrites, and 8-iso-PGF2α, respectively, were measured. The results indicated that the whole Formula was the most effective in contrasting the upregulated LDH and nitrite levels in HypoE22 cells and isolated rat striatum specimens, respectively (Figures 2B and 3A,B).

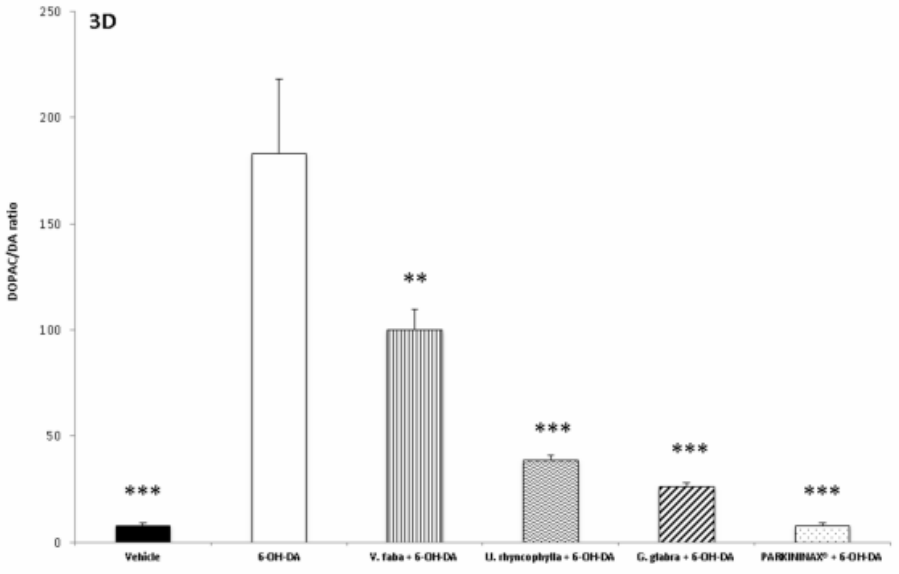




Results and discussion

The single extracts and the formula displayed comparable blunting effects on the 6-OH-DA-induced level of 8-iso-PGF2α (Figure 3C). In the same condition, we also measured the turnover of DA, expressed as the DOPAC/DA ratio. The results depicted in Figure 3D indicate that the V. faba extract was the least effective in preventing the 6-OH-DA-induced turnover of DA, whereas G. glabra and U. ryncophylla displayed comparable efficacy. On the other hand, the formula was the most effective in inhibiting DA turnover in rat striatum.





Conclusions

Concluding, the present study investigated the protective effects exerted by the water extracts of G. glabra, U. rhyncophylla, and V. faba in an experimental model of PD ex vivo. Our findings showed that all tested extracts were able to contrast the 6-OH-DA-induced increase in pro-oxidant biomarkers and DA turnover in the striatum, which could be related to their content in phenolic compounds. As a final remark, the present findings corroborate the rational for the traditional use of V. faba, G. glabra, and U. rhyncophylla extracts, as well as of their pharmacological association in order to improve their protective effects, including the increase in striatal steady levels of DA, which could be crucial in the management of the clinical symptoms of PD. In this regard, future in vivo studies prove necessary in order to verify the efficacy of these extracts.

Acknowledgments

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