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Research Article
In vitro Neuroprotective Effects of Seven Natural Products Against Rotenone-induced Toxicity in a SH-SY5Y Neuroblastoma Cells Model for Parkinson's Disease

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Abstract

Background: The mitochondrion is an essential organelles for producing most of the energy in the cell and also involved in a number of cellular activities in the cell. It plays an important role in the aging process. Mitochondrial dysfunctions are supposed to be responsible for many neurodegenerative diseases as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis (ALS). A growing body of evidence suggests that defects in mitochondrial metabolism and particularly of electron transport chain may play a role in neurodegenerative diseases. Secondary effects as proapoptotic factors and Reactive Oxygen Species (ROS) can be an early stage of several mitochondrial disorders. **Objective:** To evaluate the mitochondrial membrane potential, ROS generation, GSK-3β and CK-1δ activities of seven natural products (isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin) by using well established assays *in vitro* assays. **Materials and Methods:** In this study, neuroprotective effect of seven natural products on rotenone-induced toxicity in a SH-SY5Y neuroblastoma cells model for PD. **Results:** In this study, seven natural products were assessed the mitochondrial membrane potential, ROS generation, GSK-3β and CK-1δ activities using well established assays. The most effective compound to prevent mitochondrial membrane depolarization and decrease the ROS levels was luteolin followed by isoquercetin and astragalin. Isoquercetin, astragalin, hesperetin and rutin have good activity against GSK-3β and no activity against CK-1δ. **Conclusion:** This study results suggest that isoquercetin and astragalin exerts its neuroprotective effect against rotenone due to its mitochondrial membrane potential, ROS generation, GSK-3β and CK-1δ activities.

Key words: Natural products, mitochondrial membrane potential, reactive oxygen species, GSK-3β activities, CK-1δ activities

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Parkinson's Disease (PD) is one of the most common neurodegenerative disorders and is characterized by the selective loss of dopaminergic neurons in the substantianigra pars compacta (SNpc). The most common sporadic form of PD seems to be a complex multifactorial disorder with variable contributions of environmental factors and genetic susceptibility. The mechanism of SNpc cell death in PD is not completely known, but some of studies indicate that mitochondrial dysfunction is the primary cause because of its role in ATP (energy) production and involved in a number of biochemical process in the cell[1]. Mitochondrial dysfunction with complex I deficiency and impaired electron transfer in the substantianigra in PD have been reported[2,3].

Increasing evidence suggest that mitochondrial dysfunction and oxidative stress play a crucial role in the majority of neurodegenerative diseases, so decreased mitochondrial complex I activity have been reported in the substancianigra of postmortem brain samples from neurodegenerative diseases patients[4]. This study is focused in Parkinson's disease, using as a model cells damaged with rotenone. Exposure to rotenone reproduced a PD-like syndrome with selective loss of dopaminergic neurons[5,6]. Rotenone effects on mitochondria are energy depletion and increased ROS production[7]. Selective damage to mitochondrial complex I with a concomitant increase in ROS within the dopaminergic neurons in the substantianigra and an increase in cell death is believed to be a major causative event in PD[8,9]. Abnormalities in neuronal processes can lead to profound depolarization of $\Delta\Psi m$ and increase IROS.

Also, it is known that several protein kinases are deregulated and/or implicated in the pathogenesis of CNS disorders although, they have not been validated as therapeutic targets. In this study, two kinase involved in Parkinson's disease, GSK-3β and CK-1δ.

Excessive activation of GSK-3β activity has been linked to a number of pathological diseases including neurodegenerative and psychiatric disorders[10–13]. The hyperphosphorylation of tau (Tauopathy) is known mainly as an Alzheimer Disease (AD). Recent studies showed that tau participates significantly in PD[14-16]. Identifying new inhibitors for GSK-3β and CK-1δ activity could be a promising approach in treatment of PD.

Protein kinase casein kinase-1δ (CK-1δ) is a serine/threonine kinase that is ubiquitously expressed in eukaryotic organisms. The CK-1δ is involved in diverse biological functions such as membrane transport, regulation of DNA repair, cellular morphology, modulation of the Wnt/β-catenin and Hedgehog pathway during development and regulation of circadian rhythms[17,18]. The CK-1δ dysregulation contributes to the pathogenesis of many diseases including neurodegenerative diseases[19].

Based on the reduction of mitochondrial oxidative stress may prevent or slow down the progression of these neurodegenerative diseases and the fact is that protein kinases play a crucial role in neurodegenerative diseases including in PD and AD. In this study, neuroprotective effect of seven natural products (isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin as shown in Fig. 1 on rotenone-induced toxicity in a SH-SY5Y neuroblastoma cells model for PD by testing its effect on rotenone-induced ROS generation, mitochondrial damage, cell viability and kinases inhibition (GSK-3β and CK-1δ) in neuroblastoma SH-SY5Y cells. These compounds are common flavonoids that exist in many types of plants including fruits, vegetables and medicinal herbs.

MATERIALS AND METHODS

Chemicals: Human recombinant GSK-3β and human recombinant CK-1δ were purchased from Millipore (Millipore Iberica S.A.U.) The pre-phosphorylated polypeptide substrate GS-1 was synthesized by American Peptide Company (Sunnyvale, CA). The JC-1 dye was purchased from Tebubio Spain (Innovative Lab Services and Reagents). The C-DCDHF-DA, MTT, DMSO, fluorescein, APPH, trolox, casein, ATP and all other buffer reagents were from Sigma-Aldrich (St. Louis, MO). Kinase-Glo luminescent kinase assay was obtained from Promega (Promega Biotech Ibérica, SL). The pure compounds (isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin) were obtained from ChromaDex, USA.

Cell culture: The SH-SY5Y cells, a human dopaminergic neuroblastoma cell line were obtained from American Type Culture Collection (Rockville, MD, USA) and grown in DMEM+F12 supplemented with 10% FBS, 2 mM L-glutamine, penicillin/streptomycin (100 U mL^{-1}), 1mM sodium pyruvate and 0.1 mM non-essential amino acids (Invitrogen) at 37°C under a humidified atmosphere of 95% air and 5% CO_2. In all assays, the test compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the cells at a final concentration of 0.5% (v/v) DMSO. Previous DMSO curves were tested and non-significant cytotoxicity was observed in this concentration.

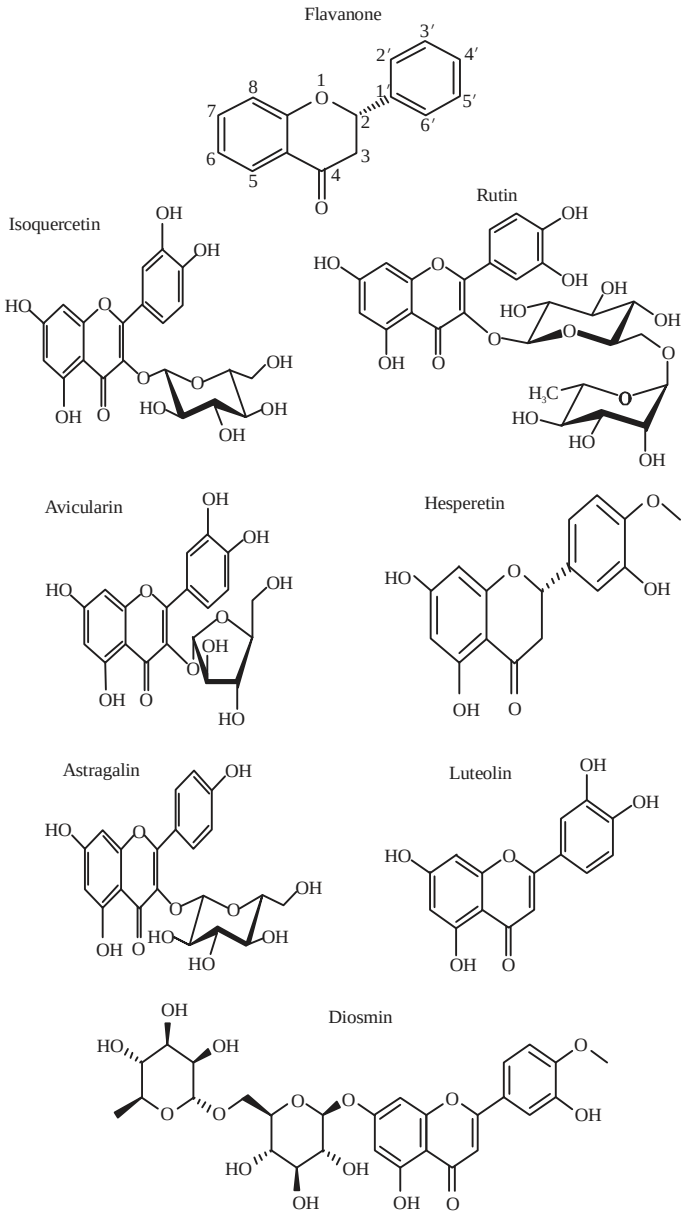

Fig. 1: Structure of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin

MTT assay: The MTT reduction rate is an indicator of the functional integrity of the mitochondria hence, of cellular viability[20,21]. For the assay, the number of cells seeded per culture well was 20000 cells per well. A total of 3 µL of each pure compounds concentration was dispensed into 600 µL of fresh medium. From this mixture, 200 µL were added to three different cell plates at a final maximum concentration of compound per well of 50 µM, 7 points, 1:2 dilution and 1% DMSO to minimize any solvent toxicity background. After 3 h of pre-treatment with compounds, cells were damaged with 1 µM rotenone and incubated 24 h. The MTT test was performed and read on Victor2^{TM} equipment.

Measurement of mitochondrial membrane potential ($\Delta\Psi m$) levels: The fluorescent dye JC-1 is situated in interior mitochondrial membrane, where it depending on the mitochondrial membrane potential ($\Delta\Psi m$) could be as monomers or aggregates[22]. At high $\Delta\Psi m$, JC-1 forms aggregates in the mitochondria that exhibit red fluorescence. At lower $\Delta\Psi m$, a lower quantity of the dye could enter into the mitochondria, no aggregates could be created and the dye maintain it monomeric form that present green fluorescence. Cells were pre-treated with different concentrations of compounds (50, 25, 12.5, 6.25 and 3.12 µM) during 3 h after that, cells were damaged with 1 µM rotenone during 24 h.

Cells were incubated with JC-1 at a final concentration of 10 μM in assay buffer (NaCl 165 mM, KCl 4.5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, Hepes 10 mM and glucose 10 mM, pH 7.4) for 30 min at 37°C. Plates were read in a FLIPR TETRA (molecular devices).

Measurement of Oxygen Radical Absorbance Capacity (ORAC): The ORAC assay is based upon the inhibition of the peroxylradical-induced oxidation initiated by thermal decomposition of azocompounds such as [2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH)][23]. The assay starts with the addition of 60 μL of a fluorescein 10 nM solutions to a black 384 plate followed by incubation for 30 min at 37°C and the addition 10 μL of APPH. At the end of this process, the fluorescence kinetics of the plates was read with FLIPR TETRA equipment. Trolox, a vitamin E analog was used as control and the results were reported as trolox equivalents (TE, number of times that the compound tested is more active than the control compound).

$$\text{Trolox Equivalent (TE)} = \frac{\text{Compound slope}}{\text{Trolox slope}}$$

Measurement of Reactive Oxygen Species (ROS) levels: The ROS generation was evaluated with the help of a fluorescent dye C-DCDHF-DA (Sigma)[24,25]. This fluorescent probe passes through the cell membrane easily and once inside the cell, its lipophilic blocking groups are cleaved by non-specific esterase's, resulting in a charged form that leaks out of the cells very slowly. The number of cells per culture well was 200,000 cells mL^{-1} in six wells plate. Plate cells were incubated overnight at 37°C, 5% CO_2 and then the medium was removed and replaced by 2 mL of fresh medium. Four microliter aliquots of compounds were dispensed in 2 mL of fresh medium at a final concentration of 20 μM. After 3 h, cells were treated with 1μM rotenone and incubated 24 h. Cells were incubated with an assay buffer (NaCl 165 mM, KCl 4.5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, Hepes 10 mM and glucose 10 mM, pH 7.4) containing C-DCDHF-DA at a final concentration of 10 μM for 30 min at 37°C. Tubes accuri cytometer (Becton Dickinson) measuring the intensity of C-DCDHF-DA. This intensity is proportional to the amount of ROS.

GSK3β activity assay: Assay buffer contained 50 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM EGTA and 15 mM magnesium acetate. Enzyme buffer contains the same formulation as assay buffer but include tween 20 0.001% in order to increase the stability of the enzyme.

Based on kinase-Glo system[26] and its capacity to detect the ATP present after the enzyme reaction, *in vitro* assay was developed for evaluate the inhibition of human GSK-3β. The kinase reaction consumes ATP and the remaining unused ATP used by the luciferase to catalize the reaction of transformation of bettle-luciferin into oxyluciferin and light emission was measured. In presence of an inhibitor, the enzyme do not consume ATP and the amount of remaining ATP increase that influence the increase of light generated by the luciferase.

The assays were performed in black 384 well plates, adding 10 μL of test extract and 10 μL of enzyme (10 ng) dissolved in enzyme buffer were added to each well. After 5 min, 10 μL of substrate containing 25 μM of GS-1 substrate and 1 μM ATP. After 30 min incubation at 30°C, 30 μL of kinase-Glo luminescent reagent was added to each well and the luminescence was recorded using a Victor2™ Wallac multimode reader.

The inhibition percentage of GSK-3β was determined by the equation:

$$\text{Inhibition percentage} = \left(\frac{RLU_{\text{Neg contr}} - RLU_{\text{Pos contr}}}{RLU_{\text{Neg contr}} - RLU_{\text{Pos contr}}}\right) \times 100$$

where, $RLU_{\text{Neg contr}}$ indicates the relatives luminiscents units recorded for negative control, RLU_{Poscontr} indicates the relatives luminiscents units recorded for positive control.

Assay buffer with 1% DMSO was used as negative control and alsterpaullone 500 nM was used as positive control. Alsterpaullone and 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (known inhibitors of the enzyme) were used as internal control curves as described earlier[27].

CK-1δ activity assay: Assay buffer contained 50 mM Hepes (pH 7.5), 1 mM NaN_3, 1 mM EGTA and 15 mM chloride acetate. The enzyme buffer contains the same formulation as assay buffer but included tween 20 0.001% in order to increase the stability of the enzyme.

The assays were performed in black 384 well plates, adding 10 μL of test compound and 10 μL of enzyme (10 ng) dissolved in enzyme buffer were added to each well. After 5 min, 10 μL of substrate containing 25 μM of casein and 3 μM ATP was dispensed. After 30 min incubation at 30°C, 30 μL of kinase-Glo luminescent reagent was added to each well and the luminescence was recorded using a Victor2™ Wallac multimode reader.

An assay buffer with 1% DMSO was used as negative control and IC-261, 10 μM was used as positive control. As

internal control curves IC-261 (know inhibitor of the ATP site of the enzyme) were used and evaluated the percentage of inhibition by using the same formula as the detection GSK-3β inhibitory activity as given in 3.7.

RESULTS AND DISCUSSION

Effects of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosminon Mitochondrial Membrane Potential (MMP): Rotenone is a naturally occurring neurotoxin and has been found to have links to Parkinson's disease[28]. In order to determine the protective effect of these compounds on rotenone-induced mitochondrial dysfunctions, $\Delta\Psi m$ was measured by JC-1.

Isoquercetin and hespertin at the highest concentration not showed protective effect on MMP, a low toxicity was detected at highest concentration with astragalin, diosmin, rutin and avicularin treatments. The best compound to prevent mitochondrial membrane depolarization was luteolin, this compound is able to maintenance of the Membrane Mitochondrial Potential (MMP) totally at 6.25 μM (totally) and partially (60%) at 3.25 μM. Isoquercetin and astragalin were able to protect totally for the rotenone effect at 25 and 12.5 μM. In the case of astragalin was able to protect 90% MMP until lowest concentration (3.12 μM). Rutin, avicularin, diosmin and hespertin presented the lowest capacity of protection over rotenone effect although, pre-treatment with all of them preserved MMP partially (Fig. 2). Mitochondrial Membrane Potential (MMP) play a significant role on neurons function and decreased MMP is strongly associated with neurodegenerative diseases including PD[29].

Effect of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin on Oxygen Radical Absorbance Capacity (ORAC): Oxygen Radical Absorbance Capacity (ORAC) has been accepted as a standard method to analyze the antioxidant potential. In this method, the peroxyl radicals react with a fluorescent compound to form nonfluorescent compound which was quantitated by fluorescence using FLIPR TETRA equipment. The antioxidant

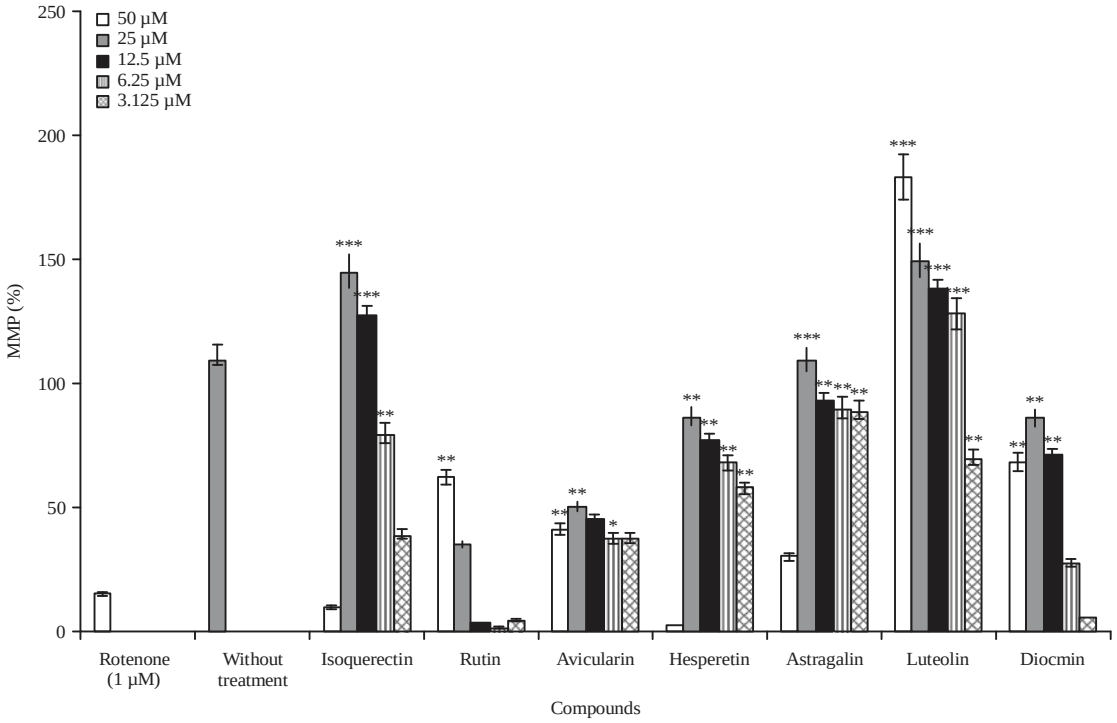

Fig. 2: JC-1 results of the different compound treatments, treatment with rotenone 1 μM decrease the MMP from 100-18%, the graphic shows neuroprotection activity against cell treated with rotenone, effect of seven natural products on rotenone-induced death cells in SH-SY5Y cells, SH-SY5Y cells were incubated with different concentration of seven natural products for 3 h and stimulated with 1 μM rotenone for 24 h at 37°C, at the end of incubation period, viability was measured with JC-1 assay, ***Significant at p<0.0005, **Significant at p<0.005, *Significant at p<0.05 versus rotenone-treated cells by Student's test and values are shown as the Mean±SD of three independent experiments

capacity was estimated by an inhibition rate and amount of product formed over time[30]. The antioxidant capacity of natural products compounds measured using the ORAC assay is shown in Table 1. The most potent antioxidant activity was detected with avicularin and isoquercetin treatment following by rutin. Hesperetin, luteolin and astragalin showed moderate activity and the less activity was showed by diosmin. The results indicate that the hydroxyl groups at positions 3' and 4' are critical for higher ORAC activity than the hydroxyl groups at position 5' and 7'.

Although, seven compounds showed different antioxidant activity, all of them was higher 2x trolox equivalent, so all of them had more antioxidant activity than trolox.

Effect of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin on rotenone-induced ROS generation: The productions of excess of free radicals which mainly include oxide, hydroxyl, hydroperoxyl, peroxy, alkoxyl radicals and the other non-free radicals are constantly produced in the human body during cell metabolism. These reactive molecules are called Reactive Oxygen Species (ROS). The ROS are toxic to the living cells[30] and can cause oxidative damage to the biological molecules[31]. This oxidative damage/stress is closely linked with pathology of many neurological diseases including PD and AD[32,33]. Several neurological disorders are associated with mitochondrial dysfunction and increase the production of free radicals species[1]. This study has used rotenone because of a strong link between rotenone exposure, mitochondrial dysfunction and PD[34]. The generation of Reactive Oxygen Species (ROS) in cell cultures upon pre-treatment with the compounds and damaged with rotenone was measured by using fluorescence dye C-DCDHF-DA using flow cytometry. In Fig. 3, treatment of the cells with rotenone increased significantly the levels of ROS in comparison to the untreated controls. Treatment with the natural products at 20 μM lowered rotenone-induced ROS at background level (DMSO 0.5%). In Table 2, 60% of cells treated alone with 1 μM rotenone were damaged compare to treatment with the present study compounds at the concentration of 20 μM (cells damaged in the range of 1.7-8.6%). The results strongly support that these compounds have the ROS inhibition properties.

Effect of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin in cell viability: In order to study the activity of the natural products in cells, the final viability effect of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin were examined in human neuroblastoma SH-SY5Y cells using the MTT test. As a result, treatment with induced death cells (100%) was compared with vehicle-treated cells (DMSO 1%) . When the cells were pre-treated with compounds (50, 25, 12.5, 6.25, 3.1, 1.56, 0.78 μM) 3 h later 1 μM rotenone was added and all compounds significantly suppressed cell death in a dose-dependent manner (Fig. 4). Treatment with isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin alone did not affect neurotoxicity (data not showed).

At higher concentration all compounds inhibit the effect of rotenone, the less potent compound was isoquercetin. In general, luteolin, diosmin and hesperetin were the most active compounds, blocked total or partially the rotenone activity at all concentration tested followed by astragalin, avicularin and rutin, blocked total or partially the rotenone activity at concentration higher than 1.56, 3.12 and 6.25 μM, respectively.

Effect of isoquercetin, rutin, avicularin, hesperetin, astragalin, luteolin and diosmin on kinases inhibition (GSK-3β and CK-1δ): The inhibition data of the both kinases were so different with the present study compounds and presented the results in Table 3. These two kinases are strongly associated with PD and other neurological disorders as reviewed earlier[35]. The astragalin, isoquercetin and luteolin are the best inhibition compounds followed by rutin. The

Table 1: Data of ORAC assay and TE

Compound ID	TE	ORAC
Astragalin	5.45±0.92	Moderate active
Avicularin	20.88±3.51	Very active
Diosmin	2.52±0.56	Less active
Hesperetin	8.60±2.34	Moderate active
Isoquercetin	19.78±3.65	Very active
Luteolin	5.70±1.21	Moderate active
Rutin	10.09±2.69	Very active

ORAC: Oxygen radical absorbance capacity and TE: Trolox equivalent

Table 2: Percentage of cells in each condition with a low level of ROS (healthy cells) and high level of ROS (damage cells)

	Cells (%)	
Compound ID	Health	Damage
Astragalin	93.2±5.3	6.8±1.1
Avicularin	91.8±6.1	8.2±2.3
Diosmin	96.2±5.5	3.8±1.4
Hesperetin	93.9±5.8	6.1±2.1
Isoquercetin	98.3±4.9	1.7±0.7
Luteolin	91.4±7.2	8.6±1.8
Rutin	91.7±6.3	8.3±1.9
DMSO (0.5%)	98.2±5.6	1.8±0.6
Rotenone (1 μM)	39.5±6.5	60.5±5.4

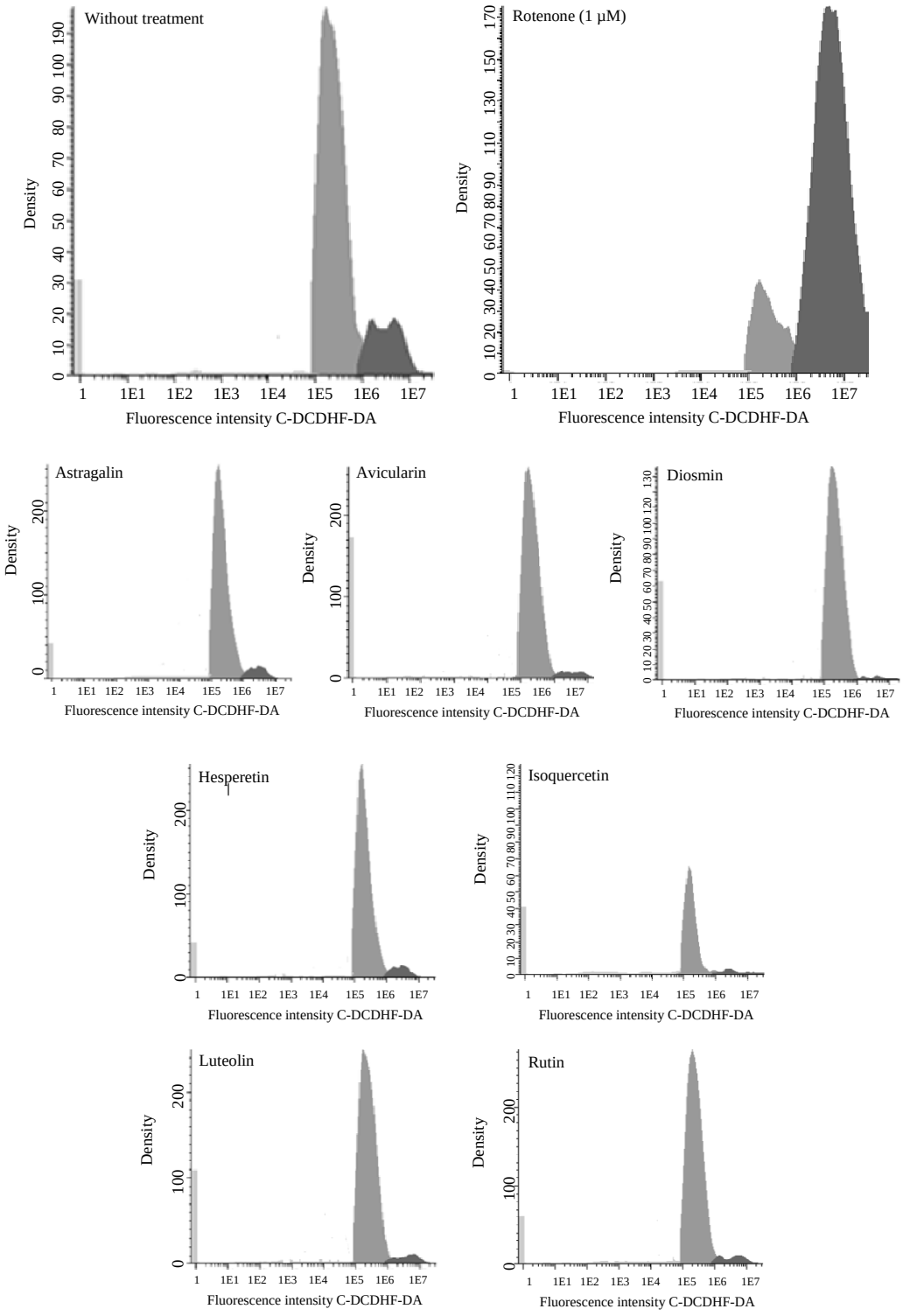

Fig. 3: Measurement of Reactive Oxygen Species (ROS) levels using flow cytometry

least inhibition compound is diosmin. Only four compounds (astragalin, avicularin, isoquercetin and luteolin) showed dual inhibition activity against both GSK-3β and CK-1δ. Astragalin, isoquercetin and luteolin are the most potent and selective GSK-3β inhibitors. In general, the activity of the flavonoids depends on the position of the hydroxyl groups, a double

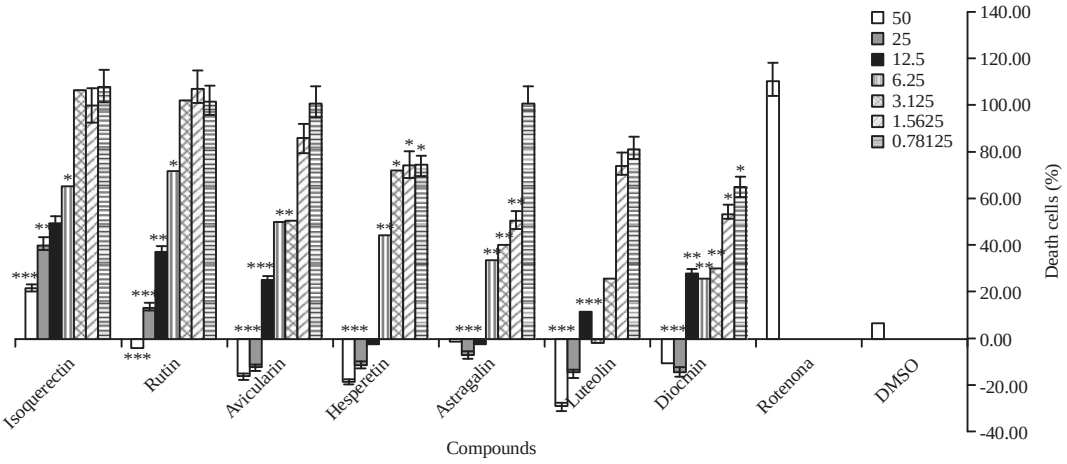

Fig. 4: Data of MTT assay, effect of seven natural products on rotenone-induced death cells in SH-SY5Y cells, SH-SY5Y cells were incubated with different concentration of seven natural products for 3 h and stimulated with 1 μM rotenone for 24 h at 37°C, at the end of incubation period, viability was measured with MTT test, ***Significant at p<0.0005, **Significant at p<0.005, *Significant at p<0.05 versus rotenone-treated cells by Student's test and values are shown as the Mean±SD of three independent experiments

Table 3: Data of kinases inhibition (GSK-3β and CK-1δ) and data shows IC_{50} (μM)

	IC_{50} (μM)	
Compound ID	GSK-3β	CK-1δ
Astragalin	1.26±0.51	72.30±9.81
Avicularin	19.5±5.16	68.09±9.22
Diosmin	>167	>167
Hesperetin	26.7±7.16	>167
Isoquercetin	1.68±0.55	84.90±10.54
Luteolin	1.94±0.63	28.80±5.9
Rutin	5.10±1.05	>167

bonds between carbon atoms (C2 and C3), a keto group and glycosylation of hydroxyl group at C3[35,36]. Several studies demonstrated that inhibition of GSK-3β is likely to be therapeutic for a number of neurological disorders including PD and AD[37]. Other neurological assays to be performed for complete evaluation of their therapeutic value.

CONCLUSION

The main objective of this study was to study the activity of seven natural products compounds in a multifactorial neurodegenerative disease including PD. Rotenone is an inhibitor of mitochondrial respiratory chain complex I is a useful tool to elicit models to study Parkinson's disease. The human neuroblastoma (SH-SY5Y) cell line is an optimal *in vitro* model to study neurodegenerative diseases including PD. These cells produce symptoms like those of many aspects of the dopaminergic neuron death observed in PD when treated with neurotoxins such as rotenone. Thus, suppressants or inhibitors of rotenone effects could be considered as a potential agents for chemopreventive and chemotherapeutic agents.

The present study compounds showed different biological activities although, all of them showed neuroprotective activity at a concentration higher than 6.25 μM, not all of them presented the same mechanism of action or the same potency.

Luteolin was the compound that offers the best profile against PD, it inhibits GSK-3β and CK-1δ, maintenance the MMP after damaged with rotenone and decreases the ROS levels. This is because of luteolin has a ortho-dihydroxyl groups at C3 and C4 and has a double bond between C2 and C3 without glycosylation.

Isoquercetin and astragalin show similar profiles, it has good activity against GSK-3β but the IC_{50} in CK-1δ assay was high. Both of them protect mitochondria of the rotenone damaged and the results indicate that both compounds reduced the ROS levels.

Hesperetin protect more than 50% of the cells at all the concentration although, it has only a medium potency against GSK-3β and no activity against CK-1δ. Due to its ability to preserve mitochondria, it was able to decreased the ROS levels.

Diosmin didn't present activity against kinases but at 25 and 12.5 μM, it shows neuroprotection activity at mitochondrial level so, it could be considered as a good antioxidant agent.

Although, avicularin inhibited both kinases, it doesn't have high potency in neuroprotection because it is able to

preserve only the 40% of the MMP although, it has a good antioxidative activity at 20 μM. Perhaps, it could be explained because it doesn't enter into the cells very good.

Rutin shows a good antioxidant profile at 20 μM but at lowest concentration it is not able to preserve the mitochondria. It is a good GSK-3β inhibitor although, no activity was detected in CK-1δ assay.

In the case of astragalin, avicularin and isoquerectin although, inhibit both kinases but the neuroprotection effect is lower. Other pathways/bioassays to be studied for evaluating the therupatic value of these compounds.

The case of diosmin showed that having only antioxidant effect, however it is able to maintain the cell alive as showed in MTT assay. More studies are necessary in order to know the mechanism of action of this compound.

In conclusion, the results showed that these natural products, markedly suppressed rotenone-induced damage in human neuroblastoma SH-SY5Y cells by inhibiting GSK-3β, attenuating ROS generation and decreasing $\Delta\Psi m$ thus, inducing a reduction in the cell death. Although, more studies are necessary, the results obtained in this study demonstrate that these compounds may be promising lead molecules for Parkinson's disease.

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