Effects of *Hypericum perforatum* extract on 6-hydroxydopamine neurotoxicity in differentiated SH-SY5Y cells

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Background and objective
Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. In our study, PD model was created as a result of exposure to 6-hydroxydopamine (6-OHDA) in SH-SY5Y cells, which is a human neuroblastoma cell line. The protective effect of *Hypericum perforatum* on PD was investigated.
Materials and methods
Phytochemical analysis of *H. perforatum* extract was performed. Then, SH-SY5Y cells were differentiated using retinoic acid and then administered 6-OHDA neurotoxin. To determine the protective effects of *H. perforatum* extract, we investigated the changes in the mRNA expression level of caspase-3, total oxidant status, and antioxidant levels in differentiated SH-SY5Y.
Results and conclusion
According to our results, *H. perforatum* extract contains glycosides, tannins, flavonoids, and carbohydrates as the major secondary metabolites. *H. perforatum* extract significantly reduced caspase-3 gene expression against 6-OHDA toxicity in differentiated SH-SY5Y cells. It was found that total oxidant status level increased significantly in the 6-OHDA experimental group compared with the control and *H. perforatum* experimental groups.
It was found that *H. perforatum* extract has an inhibitory effect on caspase-3 gene expression, which plays an important role in apoptosis. Therfore, *H. perforatum* extract has been shown to have a therapeutic potential against 6-OHDA toxicity.

Keywords:
6-hydroxydopamine, *Hypericum perforatum*, Parkinson's, disease, retinoic acid, SH-SY5Y cell line

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the formation of Lewy bodies, which are composed of aggregated alpha-synuclein protein [1,2].

To study neurotoxicity and evaluate the effectiveness of therapeutic agents, a common practice is to use 6-hydroxydopamine (6-OHDA), a hydroxylated analog of dopamine, as a toxin in PD models [3,4].

Hypericum perforatum plant has been found to contain various pharmacologically and biologically active compounds [5–7]. Studies have suggested that *H. perforatum* extract may have therapeutic potential in the treatment of PD [8,9].

This study aimed to investigate changes in the mRNA expression levels of caspase-3, as well as total oxidant and antioxidant levels, in SH-SY5Y cells differentiated with retinoic acid and treated with 6-OHDA neurotoxin.

Materials and methods

Preparation of *Hypericum perforatum* extract

H. perforatum plants that were collected from Ege University's Bornova Campus in the Izmir Province of Turkey have been identified by Asst. Prof. Dr. A. Fahri Pirhan from the Ege University Faculty of Science, Department of Biology. The flower parts of the plant were dried at room temperature before being extracted using a Soxhlet apparatus with methanol. The methanol extraction was performed as described in a previous study by Deveci *et al.* [10].

Phytochemical screening

The presence of phytochemicals was analyzed in the *H. perforatum* extract. For the screening of alkaloids, saponins, tannins, glycosides, phenols, carbohydrates,

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proteins, and flavonoids, the method established by Khuda *et al.* [11] was used.

Differentiation of SH-SY5Y human neuroblastoma cells

SH-SY5Y cells were obtained from American Type Culture Collection (ATCC). The cell passage was performed according to the method described in a previous study by Kalkan *et al.* [12].

To differentiate 75% confluent SH-SY5Y cells, after 24 h culturing the cells, the ratio of fetal bovine serum in the DMEM culture medium was reduced from 10 to 5% and differentiation was initiated by adding 10 μM retinoic acid (EMD Biosciences cat# 554720, Darmstadt, Germany). On the fourth day, the fetal bovine serum rate was reduced to 2.5%, and then on the 7th day, it was reduced to 1%. After 3 days, the experimental groups were formed [12].

Preparation of experimental groups

On 10th day of differentiation of SH-SY5Y cells, only the medium was placed in the first group as control. To induce the *in vitro* model of PD as the second group, 6-OHDA (Sigma H116) was adjusted to a final concentration of 100 μM [13].

H. perforatum extract was added at a dose of 20 μg/ml to the third group and after 24 h of incubation, 6-OHDA was adjusted to a final concentration of 100 μM. To the fourth group, at a dose of 20 μg/ml *H. perforatum* extract was added [14].

RT-PCR experiments

RNA isolation from the experimental groups was performed using the Zymo Quick RNA Miniprep Kit (Cat no: R1054). At the end of the isolation process, 30 μl of nuclease-free water was added, and the RNAs were stored at −80°C. To determine the expression levels of the caspase-3 gene for each group, the GoTaq 1-Step RT-qPCR System Technical Manual kit was used. The glyceraldehyde-3-phosphate dehydrogenase gene was used as a reference gene for normalization [15].

Total antioxidant status measurement

Total antioxidant status levels of experimental groups' supernatants were measured using a Rel Assay Diagnostics kit (Cat no: RL0017), according to the manufacturer's instruction. The color intensities of the samples were measured at 660-nm absorbance. Trolox, which is a vitamin E analog, was used as a stable antioxidant standard solution. The results obtained were expressed in μmol/l.

$$\text{Results} = \frac{[\Delta\text{Abs Sample}]}{[\Delta\text{Abs Standard}]} \times 10^{*}$$

(*= Concentration of standard).

Total oxidant status measurement

Total oxidant status level measurements of the samples' supernatants were made with a Rel Assay Diagnostics kit (Cat no: RL0024), according to the manufacturer's instruction. The color intensities of the samples were measured at 530-nm absorbance. The assay was calibrated using a hydrogen peroxide standard solution. The results obtained were expressed in μmol/l.

$$\text{Results} = \frac{[\Delta\text{Abs H2O} - \text{nAbs Sample}]}{[\Delta\text{abs H2O} - \text{nAbs Standard}]}$$

Statistical analysis

Data were expressed as mean±SD. Statistical analysis of the results was performed using the Statistical Package for the Social Sciences (SPSS, Newyork, USA) program. Statistically significant differences were analyzed using one-way analysis of variance. The differences between the groups were determined by the least significant difference test (post-hoc test in analysis of variance). Significance was defined as a P value less than or equal to 0.05.

Results

According to the results of the phytochemical screening, it was found that the *H. perforatum* extract contains glycosides, tannins, flavonoids, and carbohydrates as the major secondary metabolites (Table 1).

It was observed that the toxicity induced by 6-OHDA significantly increased the expression of the caspase-3 gene by 4.805±0.957-fold in the cells. However, the *H. perforatum* extract was found to significantly reduce the expression of the caspase-3 gene in the face of 6-OHDA toxicity in the differentiated SH-SY5Y

Table 1 Phytochemical screening of the *Hypericum perforatum* extract

Phytochemicals	Chemical tests	Results
Alkaloids	Hager's test	–
Saponins	Foam test	–
Flavonoids	General test	+
Tannins	Alkaline Reagent test	+
Glycosides	Keller-Kiliani test	+
Carbohydrates	Benedict's test	+[a]
Proteins	Xanthoproteic test	–

[a]Green (0.1–0.5% sugar).

cells. In the experimental group, a change of 0.905 ±0.281 was found in the expression of the caspase-3 gene in comparison with the control group. Furthermore, when differentiated SH-SY5Y cells were incubated only with the *H. perforatum* extract, the fold change in the expression of the caspase-3 gene was found to be 0.704±0.049 in comparison with the control group (Fig. 1).

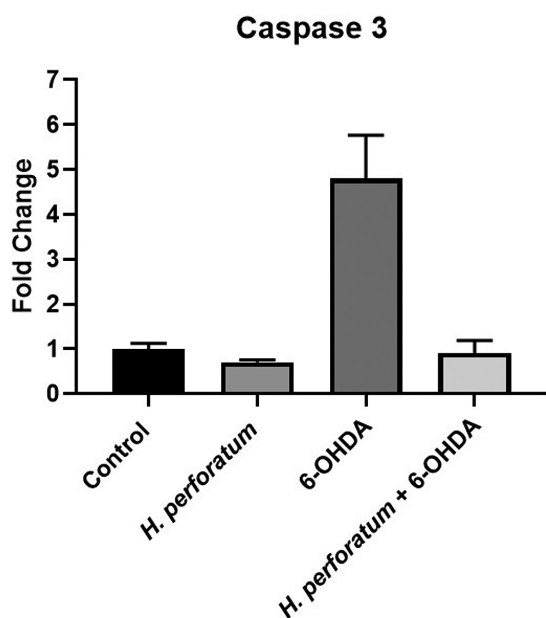
In the study, it was observed that the level of total oxidant status increased significantly in the 6-OHDA experimental group in comparison with the control and *H. perforatum* experimental groups (Fig. 2). However, it has been reported that there is a decrease in the level of total antioxidant status, but no significant results were obtained. No significant changes were observed in the other groups. In the study, no significant results were obtained as a result of the application of *H. perforatum* and in the other groups (Fig. 3).

Discussion

The neuroprotective effects of flavonoids and tannins have been demonstrated in various studies [16,17]. Hesperidin, in particular, has been shown to have multiple beneficial pharmacological effects such as anti-oxidation, anti-inflammation, and neuroprotection [18]. The main active compounds found in the *H. perforatum* plant consist of naphthodianthrone derivatives, particularly hypericin-pseudohypericin and hyperforin, which is a phloroglucinol derivative, as well as flavonoids [7]. However, the effects of specific tannin compounds and their combinations with flavonoids on the content of *H. perforatum* are currently unknown.

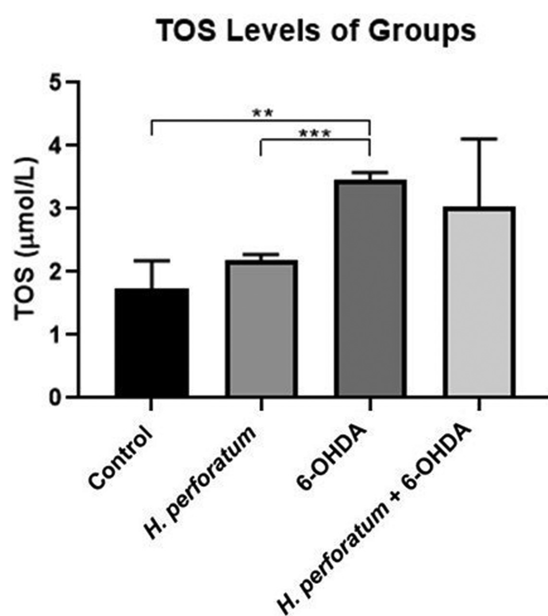
In a recent study, it has been reported that increased caspase-3 activation and expression confirm the role of apoptosis in PD [19]. Additionally, another study has reported that p38 activation underlies the toxic effects of 6-OHDA on dopaminergic neurons and that activation of the p38 signaling pathway promotes

Figure 1



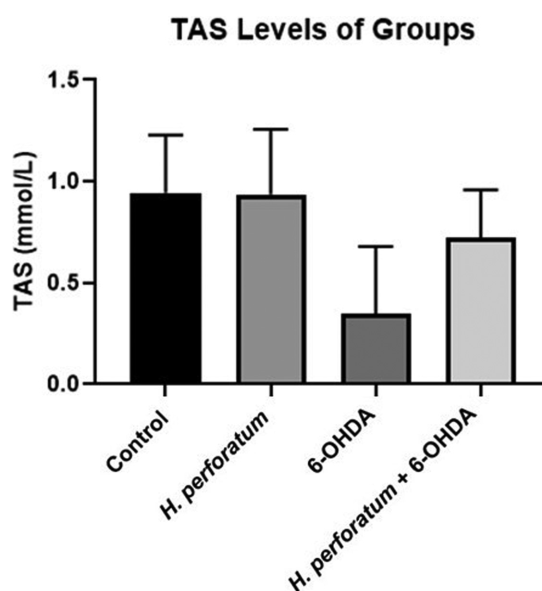
Caspase-3 gene expression alterations among experimental groups.

Figure 2



Total oxidant status levels in control of the experimental groups.

Figure 3



Total antioxidant status levels in control of the experimental groups.

cell apoptosis, a process known to be involved in PD and plays a role in neuroinflammation [20,21]. The findings obtained in these studies suggest that the content of *H. perforatum* extract has the potential as a promising new molecule for the treatment of PD.

In a study conducted by Gomez del Rio *et al.* [8], it was shown that a hyperforin-rich *H. perforatum* extract prevents dopaminergic neuron loss in experimental animals in a Parkinson's model created with rotenone. Various studies have also shown that 6-OHDA stimulation increases p38 phosphorylation and caspase-3 activation [22,23].

Conclusion

According to the results of our study, it was found that the *H. perforatum* extract has an inhibitory effect on caspase-3 gene expression, which plays an important role in apoptosis. Therefore, it has been demonstrated that *H. perforatum* extract has therapeutic potential against 6-OHDA toxicity. However, further research is necessary to fully understand their specific and combinatorial roles in neuroprotection by isolating the tannins and flavonoids found in the extract.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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