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Study the Neuroprotective Effects of Ethanoic extract of propolis on SH-SY5Y cell line Model of Parkinson's Disease

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ABSTRACT

Background: Parkinson's disease (PD) is the second most prevalent neurodegenerative disease. There are currently more than five million PD patients worldwide. Up to date, no curing treatment is available. The main characteristics of PD are the degeneration of dopaminergic neurons in the substantia nigra pars compacta and the lack of dopamine in the striatum, which result in the clinical features including tremor, bradykinesia, and muscle stiffness.

Objective: This study aims to evaluate the neuroprotective activity of *propolis ethanolic* extract (PEE) in rotenone induced Parkinson models in SH-SY5Y cells and study the immunological and biochemical effects of (PEE) on the levels of IL-6, MDA, Dopamine and TAOC and identify the doses that effectively decrease the levels of IL-6 and MDA in SH-SY5Y cells.

Materials and methods: SH-SY5Y cell lines were seeded in 96 tissue culture plates. All cells were pretreated with different concentrations of propolis ethanolic extract (PEE) at serial dilutions ranging from 31.25 to 1000 µg/ml (four replicates were used for PEE) for 2 hours at 37C then challenged with ROT (20 µg/ml), the plate was covered with a self-plastic lid. After a 48-hour incubation period following the exposure period, the cell lines were taken for immunoassay by ELISA method using MDA, IL-6, TAOC and dopamin Colorimetric assay.

The results: The result shows the *propolis* boosted the viability on SHSY5Y cell line due to its ability to inhibit neurite outgrowth of differentiating SH-SY5Y cells. and the neurotoxic agent rotenone shows high cytotoxic effect on SHSY5Y cell line. also (PEE) at different concentrations show a significant decreased (p value<0.05) in MDA and IL-6 levels.

Conclusion: In conclusion, this study indicating that natural compound propolis attenuated the neurotoxicity induced by rotenone in SH-SY5Y Parkinson's disease cell model due to its anti-inflammatory and antioxidant properties.

Keywords: Propolis; Parkinson's disease; Rotenone; Glutathione

Introduction

Chronic neurodegenerative disorder. Parkinson's disease (PD) is the second an age-related neurodegenerative disorder that affects more than 6.3 million people(1). There are currently more than five million PD patients worldwide accounting for around 2% of the world's population(2) which makes it the second most prevalent neurodegenerative disease worldwide(3) . Up to date, no curing treatment is available (4) .affecting both the motor and non-motor systems of the central nervous system. The main characteristics of PD are the degeneration of dopaminergic neurons in the substantia nigra pars compacta and the lack of dopamine in the striatum (5), which result in early indicators including tremor, rigidity, bradykinesia, and difficulties in mobility(6). The SH-SY5Y human neuroblastoma cell line was one of the most used cell lines in neurosciences, either undifferentiated or differentiated into neuron-like cells(7). cells are an excellent model system for studying the effects of toxicity on both proliferating cells and differentiated cells due to its human origin, catecholaminergic (though not strictly dopaminergic) neuronal properties, and ease of maintenance(8). The SH-SY5Y is a subline of cells isolated from the bone marrow from a metastatic neuroblastoma of a 4-year-old female in1970s(9). To obtain the specific features of the diseased cells, the immortalized cell lines must be first differentiated towards a specific neuronal type that is affected in the pathophysiology of the disease. For PD, a differentiation in dopaminergic neuronal phenotype is usually achieved(7). In addition, the cell culture may be either exposed to one of the several neurotoxins such as ROT that induce cellular PD-specific changes, or be genetically modified by transfection to over-express the pathological protein (7). The cell line has a catecholaminergic phenotype, equipped with tyrosine hydroxylase and dopaminebeta-hydroxylase enzymes, and is able to synthesize both dopamine and noradrenaline neurotransmitters(10). In addition, SH-SY5Y can be further differentiated into a more mature dopaminergic phenotype(11). Thus, these characteristics make this cell line a suitable in vitro model for the study of PD. In vitro models used for PD must reproduce the two main pathologic changes of the disease: the degeneration of the dopaminergic neurons and the intraneuronal deposition of alpha-synuclein (8).

2. Materials and Methods

Cells: Human neuroblastoma SH-SY5Y cells were purchased from College of Medicine, University of Babylon, Iraq. The cells were cultured in RPMI media (Gibco/U.K.) with 10% (*v*/*v*) fetal bovine serum (FBS) (Gibco/U.K.) and 80 mg of gentamicin (The Arab Pharm/Jordan) were added. The cells were then incubated at 37 °C in a saturated humidity atmosphere containing 95% air and 5% CO_2. Cells grew to around 70% confluences before they were used for experiments. The cell lines were taken for immunoassay by ELISA method using MDA, IL-6, TAOC and dopamin Colorimetric assay.

In vitro:

Study Design

Cytotoxicity Assay of Rotenone in Cell Line

A pilot study was done to choose the appropriate concentration of ROT. In 96 tissue culture plates, SH-SY5Y cell lines were sown and marked. Four repetitions of each concentration of rotenone were employed for the Cell, and four repetitions served as the control group. The rotenone was applied to all cells at serial dilutions ranging from (0.6 to 40 µg/ml) the plate was covered with a self-plastic lid. Following a 24-hour incubation period, the proliferation of the cell lines was evaluated using an MTT assay to determine cytotoxicity(12).

Cytotoxicity Assay of Ethanolic Extract of propolis in Cell Line

In 96 tissue culture plates, SH-SY5Ycell lines were sown and marked. Four replicates of each concentration of ethanolic extract were employed, and four untreated replicates served as the control group. The extracts were added to cells at serial dilutions ranging from (31.25 to 1000µg/ml), the plate was covered with a self-plastic lid. Following a 24-hour incubation period, the proliferation of the cell lines was evaluated using an MTT assay to determine the cytotoxicity(13)

Preparation of Reagents and Solutions

Rotenone Preparation for SH-SY5Y Cells

Preparation of stock solution of rotenone by dissolving 5.6 mg of powder in 0.5 ml of DMSO to produce a final concentration of 20µg/ml. The mixture is then filtered through a 0.22 µm Millipore filter to remove any contaminants(12).

Proplis Preparation for SH- SY5Y Cells

Dry powder of propolis were collected from local markets (Babylon, Iraq) and identified by a botanist at Al-Qassim green university/college of agriculture. About 30g of propolis dry powder dissolved in 250ml of ethanol (95%) ,the extract was done using soxhlet for 4 hours and temperature ranged between 80°C and 85°C.Then a rotary flash evaporator was used to remove the solvent from the extract, and the resultant extract was stored at 20° C until use. Preparation of stock solution of ethanolic extracts of *propolis* was done by dissolving 150 mg of the dried extract in 10 ml of serum-free RPMI to produce a final concentration of 1000µg/ml. The mixture was then filtered through a 0.22 µm Millipore filter to remove any contaminant.(14)(15)

3. Results

In Vitro

Cytotoxicity Assay

Cytotoxicity Assay of Different Concentrations of Rotenone in Cell Line

There was a significant decrease (P-value<0.05) in the viability of the SH-SY5Ycells in all rotenone concentration except at (5 and 10 µg /ml) when compared with the control group after an incubated period of 24 hours at 37°C. Figure (3.1)

Effect of propolis extract on cells viability

Propolis extract significantly (P≤0.05) reduce cells viability at concentrations of (500 and 1000 µg/ml) as compared to untreated control group. As illustrated in figure 3.2

Effect of propolis – Rotenone combination on cell viability

Treatment of the cells with rotenone and different concentrations of Propolis caused significant (P≤0.05) decrease only at concentration of 31 µg/ml compared to control group. On the other hand, it caused a significant (P≤0.05) increase in cells viability when the cells were treated with Propolis extract at all concentrations used as compared to Rot group. As illustrated in figure (3.3).

Biochemical study

Effect of Propolis on (IL-6), (MDA), (DOP) and (TAOC) Levels

Treatment with Propolis extract caused significant decrease (p-value ≤0.05) in (IL-6) levels at all propolis concentrations used also caused significant decrease (p-value ≤0.05) in (MDA) levels at concentration of 500 and 1000 ug/ml as compared to control group while Treatment with Propolis extract caused significant increase (p-value ≤0.05) in (DOP) levels at concentration of 500 and 1000 ug/ml and caused significant increase (p-value ≤0.05) in (TAOC) levels at concentration of 62.5 and 125 ug/ml as compared to control group figure figure(3.4).

Effect of Propolis and Rotenone Combination on (IL-6), (MDA), (DOP) and (TAOC) Level

Treatment with (Propolis + rot) combination caused significant decrease (p-value ≤0.05) in IL-6 levels as compared to positive control at all concentration except for (1000 µg/ml) while there is no significant change as compared to negative control as well as (Propolis + rot) combination caused a significant decrease(p-value ≤0.05) in MDA levels at conc. Of (250 to1000 µg/ml) as compared to positive or negative controls. Also, this combination results in a significant increase (p-value ≤0.05) in DOP levels at conc. Of (31 and 62 µg/ml) as compared to negative control and at all concentration as compared to positive control.as well as propoli+rotenone combination causes a significant increase (p-value ≤0.05) in TAOC levels at concentration of 31 to 250 µg/ml as compared to negative and positive control. figure (3.5)

4. Discussion

4.1. In Vitro

4.1.1. Cytotoxicity Assay in SH-SY5Y Cells:

Results show that ROT at a concentration of (20 and 40 µg/ml) caused a significant decrease in the viability of SH-SY5Y cells, which agrees with the study of (16) . One common in vitro model for dopaminergic cells is the human catecholaminergic neuroblastoma cell line SH-SY5Y. Because of their high ROT sensitivity, SH-SY5Y cells were ideal for in vitro studies of rotenone (ROT) neurotoxicity(17).

The possibility that Propolis Ethanolic Extract might shield SH-SY5Y cells from the neurotoxicity that ROT induces has not been investigated experimentally. Cell viability was substantially decreased in the ROT group compared to the control group. The accumulation of synuclein, disruption of mitochondrial function, and death of neuronal cells are all consequences of ROT exposure, which also inhibits mitochondrial respiratory chain complex I (MC-1) and increases ROS production(18)..

SHSYS cells were treated with different concentrations of propolis has shown no effect on cell viability**.** Propolis inhibits neurite outgrowth of differentiating SH-SY5Y neuroblastoma cells but statically there is no significant deference on the cell viability(15). While at high concentrations of PEE (500and 1000µg/ml), cell viability decreases due to the presence of phenolic compounds (mainly flavanones and dihydroflavonols, as well as a series of esters of p-coumaric acid, ferulic acid, benzoic acid and fatty acids (palmitic acid, linoleic acid, oleic acid) that increase the cytotoxic stats(19) also The antiproliferative effects of quercetin and chyricin have been reported at high concentration , Furthermore, a higher antiproliferative effect has been shown with high phenolic content (20)(21).

substrate of Abl(22) and can be phosphorylated at Tyr221 by Ab. Abl kinase is involved in RA-mediated neuroprotective effect against rotenone, Rotenone exposure induced increases in Abl Y412 and CrkII Y221 phosphorylation, while treatment with RA significantly reduced Abl and CrkII phosphorylation and increase cell viability in cell under rotenone stress. Inhibiting Abl by (RA)reversed the decline of dopaminergic neurons and mitochondrial function in many rat models of Parkinson's disease. Chronic myeloid leukemia was the initial indication for the development of nilotinib, a powerful Abl inhibitor. In a further investigation, researchers found that nilotinib prevented rotenon-induced preclinical PD in rat by protecting dopaminergic neurons (23). Martins et al. (2021) found that the Propolis chemical reduces α-syn levels and suppresses Abl protein signaling via reducing intracellular ROS levels. AMPK activation and cell survival may increase with Abl inhibition. AMPK activation alters cellular metabolism, increases antioxidant capacity, improves mitochondrial quality control, and induces autophagy in the Parkinson's disease model. Rosmarinic acid (RA) also decreased ROT-induced ROS and restored mitochondrial membrane potential and ATP. PEE protected SH-SY5Y cells against ROT by restoring mitochondrial membrane potential, cellular ATP, and ROS turnover(24)

4.1.2. Effect of P Ethanolic Extracts on Biochemical and Immunological Parameters

4.1.2.2. Interleukin -6 (IL-6 Assay in Vitro)

According to the current work results, the rotenone (ROT) group significantly increased IL-6 compared to the control (healthy) group. ROT drastically decreased SOD, CAT, and glutathione levels. In addition, ROT raised proinflammatory cytokines (IL-1β, IL-6, and TNF-α), and inflammatory mediators (COX-2 and iNOS) (27)

In the SH-SY5Y model, we examined the neuroprotective effects of (PEE) when subjected to ROT stress. Through its antioxidant and ROS-depleting properties, (PEE) significantly mitigated ROT-induced cell death in SH-SY5Y(28). This might be due to the antioxidant components in (PEE) have been activated including non-enzymatic antioxidants like Vitamin C and enzymatic antioxidants like Catalase, which maintain the membranes of live cells(29)**.**

Carvacrol is a monoterpenoid that acts as a potent activator of the TRPV3 (Transient Receptor Potential Subtype V3) and TRPA1 (Transient Receptor Potential Subtype A1) ionic canals. These canals are capsaicin receptors that play a key role as a mediator of inflammatory pain. Carvacrol also inhibits COX-2 and has an analgesic effect (30)

PEE Products, Inc. Neuroinflammation Prevention Strategies PD research shows that central and local inflammation, typified by CD4 T cell infiltration and CD11b+ microglia/macrophages activation, contributes to neuronal death. inhibited NF-κB activation in ROT-intoxicated rats. Low levels of inflammatory markers such as TNF-α, IL-6, were observe in Chronic stimulation of these cells causes morphological and functional alterations that reduce ROS generation shows (31)**.** Recent evidence suggests that PEE products like RJ can activate NRF2, affecting neuroinflammation. In addition to promoting antioxidant release, NRF2 directly inhibits inflammatory responses by downregulating proinflammatory cytokines like IL-6 and IL-1β transcription , propolis may also reduce neuroinflammation by regulating antioxidant genes like HO-1(32) **.**

Hypoxia-inducible factor 1 (HO-1) protects cells from oxidative damage and inflammation. Degrading nonprotein-bound free heme, which is cytotoxic and proinflammatory, reduces inflammation. Carbon monoxide, which has anti-inflammatory and antiapoptotic properties, ferritin, which chelates free iron, and biliverdin, which is enzymatically reduced by biliverdin reductase into bilirubin, are formed when HO-1 degrades heme(33)**..**

4.1.2.3. Total Antioxidant Capacity Assay in Vitro

The rotenone (ROT) group significantly decreased the total antioxidant capacity (TAOC) levels as compared to the control (healthy) group, which agrees with the previous study by (34). , the midbrain, striatum, and spinal cord showed reduced TH and DAT expression, as well as altered dopamine receptor and brain-derived neurotrophic factor expression. Rotenone also caused midbrain-specific inflammatory responses with increased glial marker expression, but not in extra-nigral areas. The nigral and extra-nigral regions showed widespread mitochondrial function changes, increased oxidative stress signatures, and disruptions of neuroprotective peptides like PACAP, VIP, and ADNP. This study

confirms that systemic rotenone poisoning, like PD, generates neurochemical changes at numerous CNS levels, making this pre-clinical model suitable for studying extra-nigral abnormalities of PD(35).

According to (12), mitochondrial transmembrane potential changes and mitochondrial dysfunction were caused by rotenone-induced toxicity and ROS generation in SH-SY5Y cells. Reduced ATP production was a result of transmembrane potential disruption and mitochondrial complex-I activity(12)

When contrasted with the ROT group, PEE treatment considerably raises TAOC levels in PD patients (31 g/ml). Vitamin C, butylated hydroxytoluene, and PEE such as cinnamic, ferulic, and caffeic acids serve as primary antioxidants or free radical terminators. Polyphenols in propolis have reducing, hydrogen-donating, and singlet-oxygen-scavenging properties(36).

Propolis' main antioxidants are galanin and pinocembrin2. Phenolic components in propolis are thought to donate hydrogen ions to free radicals, preventing lipid, protein, and nucleic acid oxidation (37).

Propolis extracts directly modulated lipid peroxidation where LDL level decreased from 1.3 to 0.8 g/L after giving propolis, illustrating the antioxidant contribution of propolis. orally administered pure antioxidant compounds such as caffeic acid, quercetin, and kaempferol at the doses corresponding to the in vitro antioxidant capacity of the propolis extract did not restore the physiological parameters (38).

In addition to working in proton donation, killing free radicals and contributing through the important elements contained in the , such as cobalt, and its mechanism of action, such as vitamin C and B12, its phenolic components also work according to a double mechanism due to its active components, which work in reducing oxidants and increasing antioxidant capacity As Propolis inhibits the activity of cyclooxygenase (COX) and lipoxygenase, thereby reducing the production of prostaglandin E2 and the expression of the inducible isoform of COX-2 (39).

Hämäläinen, Nieminen et al explained that Prostaglandin E2 (PGE2) has a central role in inflammation and both cyclooxygenase-2 (COX-2) and prostaglandin E synthases are critical enzymes in its synthesis. In inflammation, bacterial products and cytokines enhance the expression of COX-2 and inducible microsomal prostaglandin E synthase-1 (mPGES-1) which are functionally coupled to result in increased PGE2 formation in macrophages and tissue cells (40)

In the present study, we systematically investigated the effects of 26 naturally occurring flavonoids on PGE2 production and on COX-2 and mPGES-1 expression in activated macrophages. Twelve flavonoids, i.e., flavone, luteolin-7-glucoside, kaempferol, isorhamnetin, morin, quercetin, naringenin, taxifolin, pelargonidin, daidzein, genistein, and genistin effectively inhibited lipopolysaccharide (LPS)-induced PGE2 production. Four flavonoids (flavone, isorhamnetin, daidzein, and genistein) inhibited significantly LPS-

induced COX-2 expression, while mPGES-1 expression was downregulated by kaempferol and isorhamnetin**.** (41)

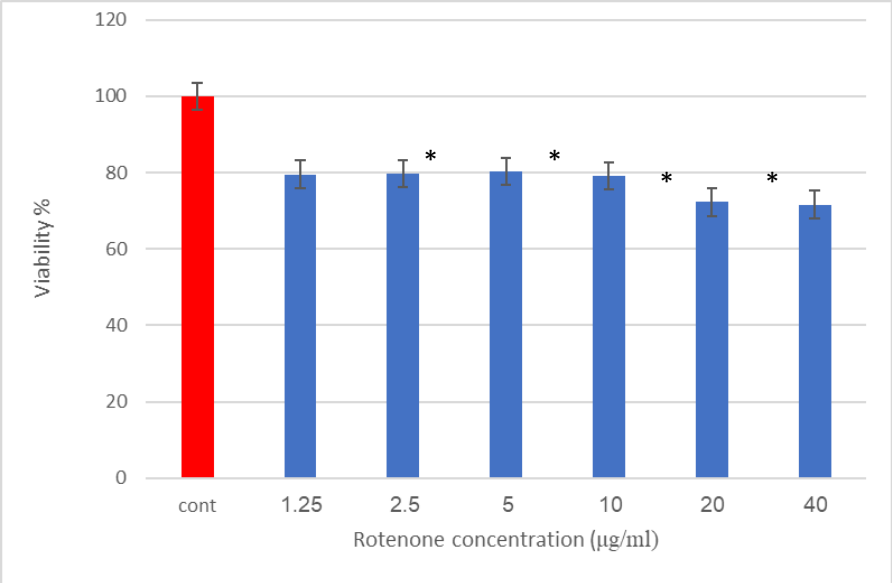
4.1.2.3. Dopamine

Administration of rotenone caused reduction in dopamine levels as compared to the control (healthy), in the striatum. acute administration of rotenone caused an increase in the GSH / GSSG ratio by 69 %. The motor stage developed after a decrease in the number of cells in the SNpc by more than 30 %, and was characterized by changes in the dopaminergic system, leading up to a 71 % reduction in dopamine levels in the striatum (42).

Oxidative stress from dopamine oxidation may trigger selective dopamine neuron loss in PD. Dopamine homeostasis is crucial to PD pathogenesis. Monoamine oxidase and spontaneous dopamine oxidation into quinine structure increase reactive oxygen species (ROS). ROS generate oxidative protein changes, inhibition of key protein activities, and altered protein breakdown, which may contribute to PD (43). After (PEE) treatment, a significant increase in dopamine levels is produced in Parkinson's affected at (31 g/ml) in comparison to the ROT group Consistent with several experimental studies Parkinson's disease models, the inclusion of propolis components resulted in an increase in dopamine levels in the substantia nigra (SNC)(44). According to Ali and Kunugi (2020), dopamine, DOPAC, and HVA levels were elevated in ROT-induced cell after the use of chrysin (44)

Because it did not alter mono amino oxidase activity or brain MPP levels, CAPE's neuroprotective effect does not reduce MPTP metabolism to MPP+ . The neuroprotective effects of chrysin and CAPE were observed to enhance dopamine production (45)**.**

Researchers found that rats exposed to neurotoxins had an increase in TH+ neurons and an improvement in neuronal survival after ingesting RJ, HPO-DAEE, and propolis flavonoids(44)



Figure(3.1) Effects of different concentrations of rotenone on the viability of SH -SY5Y cell line
*=significant decrease (p-value ≤0.05) as compared to the control group**.**

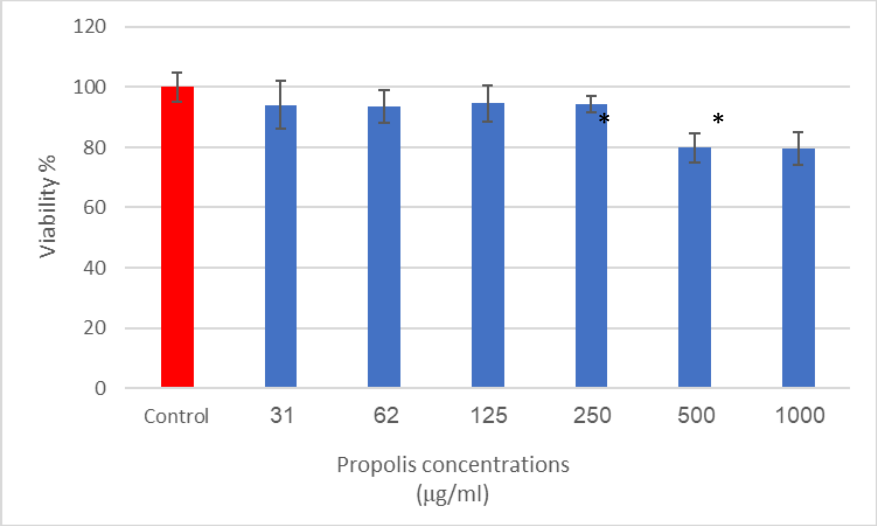


Figure (3.2) Effect of propolis extract on SHSYS cell line viability

*=significant decrease (p-value ≤0.05) as compared to the control group.

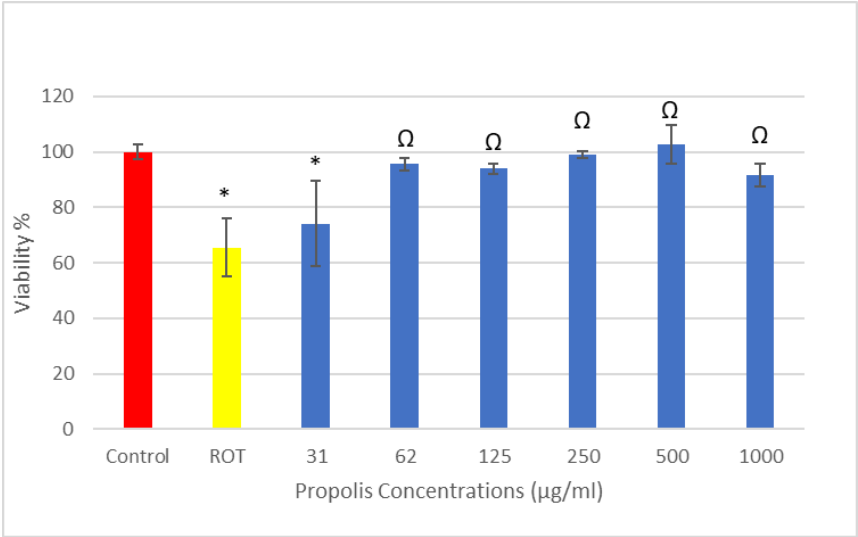


Figure (3.**3**) Effect of Propolis on the viability of SHSY cells in the presence of constant concentration of Rotenone. *=significant decrease (p-value ≤0.05) as compared to control group. Ω=significant increase (p-value ≤0.05) as compared to ROT group.

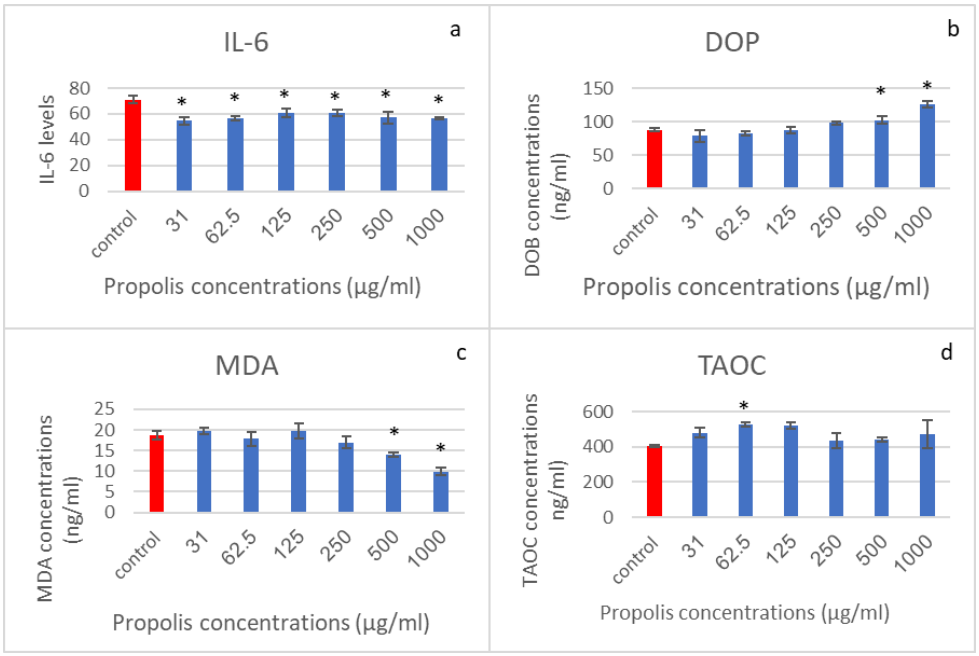


Figure (3.4) Effect of different concentrations of propolis on IL-6 levels (a), DOP (b), MDA (c), and TAOC (d). * =significant decrease (p-value ≤0.05) as compared to control group.

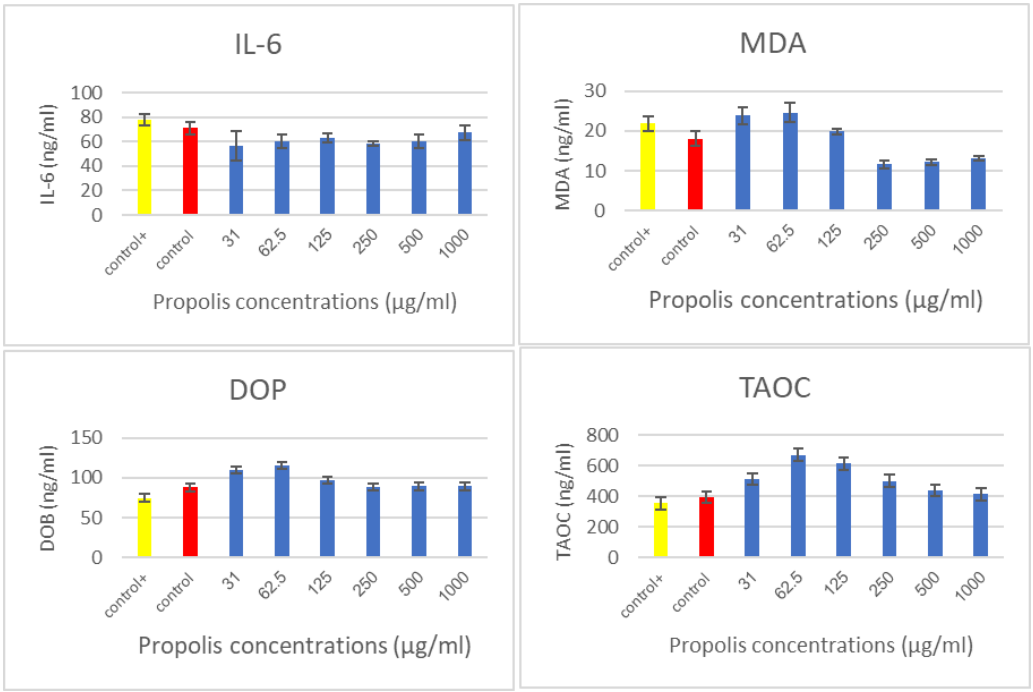


Figure (3.5): Effect of (propolis-Rotenone) combination on IL-6 (a), MDA (b) , DOB (c), and TAOC (d) concentrations. Control+: cells treated with Rotenone (20 µg/ml) only, Control: untreated cells. *=Indicate significant change as compared to negative control, # =Denotes significant change as compared with positive control.

Conclusion

In Vitro

1. In SH-SY5Y cells, the P. officinalis ethanolic extract significantly reduces IL-6. This suggests it may be an important key in slowing Parkinson's disease.

2. PEE demonstrates significant efficacy in reducing MDA levels, a highly toxic chemical found in SH-SY5Y cells. This highlights its function as an antioxidant and defense mechanism, safeguarding tissue against excessive free radicals, which are recognized as a contributing factor to Parkinson's disease.

3. PEE enhances antioxidant levels in SH-SY5Y cells, thereby safeguarding tissue against free radicals, which are recognized as a contributing factor to Parkinson's disease.

4. The administration of PEE treatment has been observed to enhance dopamine levels in SH-SY5Y cells, indicating its significant role in protecting tissue from free radicals, which are recognized as a contributing factor to Parkinson's disease.

REFERENCES:

1. Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood–brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab. 2016;36(9):1481–507.

2. Lama A, Pirozzi C, Avagliano C, Annunziata C, Mollica MP, Calignano A, et al. Nutraceuticals: An integrative approach to starve Parkinson’s disease. Brain, Behav Immunity-Health. 2020; 2:100037.

3. Awad K, Abushouk AI, AbdelKarim AH, Mohammed M, Negida A, Shalash AS. Bee venom for the treatment of Parkinson’s disease: How far is it possible? Biomed Pharmacother. 2017; 91:295–302.

4. Werner MH, Olanow CW. Parkinson’s disease modification through Abl kinase inhibition: an opportunity. Mov Disord. 2022;37(1):6–15.

5. Thomas B, Beal MF. Parkinson’s disease. Hum Mol Genet. 2007;16(R2):R183–94.

6. Titova N, Qamar MA, Chaudhuri KR. The nonmotor features of Parkinson’s disease. Int Rev Neurobiol. 2017; 132:33–54.

7. Cetin S, Knez D, Gobec S, Kos J, Pišlar A. Cell models for Alzheimer’s and Parkinson’s disease: At the interface of biology and drug discovery. Biomed Pharmacother. 2022; 149:112924.

8. Falkenburger BH, Saridaki T, Dinter E. Cellular models for Parkinson’s disease. J Neurochem. 2016; 139:121–30.

9. Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res. 1978;38(11_Part_1):3751–7.

10. Presgraves SP, Ahmed T, Borwege S, Joyce JN. Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists. Neurotox Res. 2003; 5:579–98.

11. Lopez-Suarez L, Al Awabdh S, Coumoul X, Chauvet C. The SH-SY5Y human neuroblastoma cell line, a relevant in vitro cell model for investigating neurotoxicology in human: Focus on organic pollutants. Neurotoxicology. 2022;

12. Han X, Han B, Zhao Y, Li G, Wang T, He J, et al. Rosmarinic Acid Attenuates Rotenone-Induced Neurotoxicity in SH-SY5Y Parkinson's Disease Cell Model through Abl Inhibition. Nutrients. 2022;14(17):3508.

13. Chiu HF, Han YC, Shen YC, Golovinskaia O, Venkatakrishnan K, Wang CK. Chemopreventive and chemotherapeutic effect of propolis and its constituents: a mini-review. J Cancer Prev. 2020;25(2):70.

14. Alwaeely FA, Madlum KN, Alsaadi MA. Immunomodulatory Effect of Propolis on Foxp3 Gene Expression in Human Peripheral Blood Mononuclear Cells Stimulated in vitro with Pseudomonas Aeruginosa Ag. Arch Razi Inst. 2021;76(4):887–94.

15. Kim HB, Yoo BS. Propolis inhibits neurite outgrowth in differentiating SH-SY5Y human neuroblastoma cells. Toxicol Res. 2016;32:239–43.

16. Martins JB, de Lourdes Bastos M, Carvalho F, Capela JP. Differential effects of methyl-4-phenylpyridinium ion, rotenone, and paraquat on differentiated SH-SY5Y cells. J Toxicol. 2013;2013.

17. Seoposengwe KM. The effect of selected medicinal plants on rotenone-induced toxicity in SH-SY5Y neuroblastoma cells. University of Pretoria; 2013.

18. Yang K, Zeng L, Zeng J, Deng Y, Wang S, Xu H, et al. Research progress in the molecular mechanism of ferroptosis in Parkinson's disease and regulation by natural plant products. Ageing Res Rev. 2023;102063.

19. Popova M, Giannopoulou E, Skalicka-Woźniak K, Graikou K, Widelski J, Bankova V, et al. Characterization and biological evaluation of propolis from Poland. Molecules. 2017;22(7):1159.

20. Jaganathan SK, Mandal M. Honey constituents and their apoptotic effect in colon cancer cells. J Apiproduct Apimedical Sci. 2009;1(2):29–36.

21. Kang T bang, Liang N ci. Studies on the inhibitory effects of quercetin on the growth of HL-60 leukemia cells. Biochem Pharmacol. 1997;54(9):1013–8.

22. Feller SM, Knudsen B, Hanafusa H. c-Abl kinase regulates the protein binding activity of c-Crk. EMBO J. 1994;13(10):2341–51.

23. Karuppagounder SS, Brahmachari S, Lee Y, Dawson VL, Dawson TM, Ko HS. The c-Abl inhibitor, nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. Sci Rep. 2014;4(1):4874.

24. Martins WK, da Silva M do N, Pandey K, Maejima I, Ramalho E, Olivon VC, et al. Current Research in Pharmacology and Drug Discovery. Curr Res Pharmacol Drug Discov. 2021;2:100033.

25. Liu L, Rajkumar M, Pandi B, Jayaraman A, Sonamuthu J. Combined neuroprotective potential of Levodopa and Resveratrol to ameliorates cognitive impairments in rotenone-induced Parkinson's disease via targeting TLR mediated neuroinflammation. Authorea Prepr. 2023;

26. Sofian ZM, Shafee SS, Abdullah JM, Osman H, Razak SA. Evaluation of the Cytotoxicity of Levodopa and its Complex with Hydroxypropyl-ß-Cyclodextrin (HP-ß-CD) to an Astrocyte Cell Line. Malaysian J Med Sci MJMS. 2014;21(Spec Issue):6.

27. Khazdair MR, Kianmehr M, Anaeigoudari A. Effects of medicinal plants and flavonoids on Parkinson's disease: a review on basic and clinical evidences. Adv Pharm Bull. 2021;11(2):224.

28. A. Neuroprotective effects of gemfibrozil in neurological disorders: Focus on inflammation and molecular mechanisms. CNS Neurosci Ther. 2024;30(3):e14473.

29. Andrés CMC, Pérez de la Lastra JM, Juan CA, Plou FJ, Pérez-Lebeña E. Antioxidant Metabolism Pathways in Vitamins, Polyphenols, and Selenium: Parallels and Divergences. Int J Mol Sci. 2024;25(5):2600.

30. Ahangari Z, Naseri M, Vatandoost F. Propolis: Chemical composition and its applications in endodontics. Iran Endod J. 2018;13(3):285.

31. Jin X, Liu Q, Jia L, Li M, Wang X. Pinocembrin attenuates 6-OHDA-induced neuronal cell death through Nrf2/ARE pathway in SH-SY5Y cells. Cell Mol Neurobiol. 2015;35(3):323–33.

32. Park JS, Rustamov N, Roh YS. The roles of NFR2-regulated oxidative stress and mitochondrial quality control in chronic liver diseases. Antioxidants. 2023;12(11):1928.

33. Chenet AL, Duarte AR, de Almeida FJS, Andrade CMB, de Oliveira MR. Carvacrol depends on heme oxygenase-1 (HO-1) to exert antioxidant, anti-inflammatory, and mitochondria-related protection in the human neuroblastoma SH-SY5Y cells line exposed to hydrogen peroxide. Neurochem Res. 2019;44:884–96.

34. Anghinoni JM, Birmann PT, da Rocha MJ, Gomes CS, Davies MJ, Brüning CA, et al. Recent Advances in the Synthesis and Antioxidant Activity of Low Molecular Mass Organoselenium Molecules. Molecules. 2023;28(21):7349.

35. Thomas Broome S, Castorina A. Systemic Rotenone Administration Causes Extra-Nigral Alterations in C57BL/6 Mice. Biomedicines. 2022;10(12):3174.

36. Gülçin I, Bursal E, Şehitoğlu MH, Bilsel M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem Toxicol. 2010;48(8–9):2227–38.

37. Nichitoi MM, Josceanu AM, Isopescu RD, Isopencu GO, Geana EI, Ciucure CT, et al. Polyphenolics profile effects upon the antioxidant and antimicrobial activity of propolis extracts. Sci Rep. 2021;11(1):20113.

38. Boufadi YM, Van Antwerpen P, Chikh Alard I, Nève J, Djennas N, Riazi A, et al. Antioxidant effects and bioavailability evaluation of propolis extract and its content of pure polyphenols. J Food Biochem. 2018;42(1): e12434.

39. Oršolić N, Jazvinšćak Jembrek M. Molecular and cellular mechanisms of propolis and its polyphenolic compounds against cancer. Int J Mol Sci. 2022;23(18):10479.

40. Park JY, Pillinger MH, Abramson SB. Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. Clin Immunol. 2006;119(3):229–40.

41. Hämäläinen M, Nieminen R, Asmawi MZ, Vuorela P, Vapaatalo H, Moilanen E. Effects of flavonoids on prostaglandin E2 production and on COX-2 and mPGES-1 expressions in activated macrophages. Planta Med. 2011;77(13):1504–11.

42. Ibarra-Gutiérrez MT, Serrano-García N, Alcaraz-Zubeldia M, Pedraza-Chaverri J, Orozco-Ibarra M. An exploratory study on the ability of manganese to supplement rotenone neurotoxicity in rats. Brain Res. 2024;149017.

43. Chagraoui A, Anouar Y, De Deurwaerdere P, Arias HR. To what extent may aminochrome increase the vulnerability of dopaminergic neurons in the context of Parkinson's disease. Int J Biochem Cell Biol. 2024; 168:106528.

44. Ali AM, Kunugi H. Apitherapy for Parkinson's disease: A focus on the effects of propolis and royal jelly. Oxid Med Cell Longev. 2020;2020.

45. Ibrakaw AS, Omoruyi SI, Ekpo OE, Hussein AA. Neuroprotective activities of Boophone haemanthoides (Amaryllidaceae) extract and its chemical constituents. Molecules. 2020;25(22):5376.