Antioxidant Activities of Bingöl Royal Jelly on SH-SY5Y Cells

Abstract
Royal Jelly is a bee product with high protein content is a unique nutrient for the queen honeybee. It leads to a substantial elongation of the lifetime of the queen in comparison to the worker honeybees via anti-inflammatory, anti-oxidant, anti-cancer and anti-microbial properties. Flavonoids naturally have reported to have anticancer activities thanks to their potent antioxidant activity. The antioxidant activity of RJ is attributed to its rich flavonoid content however anti-oxidant activities of the Bingöl RJ has yet to be explored in detail. The goal of the current study was to investigate the antioxidant activities of Bingöl RJ on SH-SY5Y neuroblastoma cells. The results of our study revealed that Bingöl RJ at a concentration of 0.3 mg/ml significantly augmented ROS level in SH-SY5Y cells while 0.5 mg/ml of RJ had almost no effect on ROS levels. The levels of malondialdehyde in SH-SY5Y cells considerably increased in the presence of 0.3 mg/ml RJ while 0.5 mg/ml RJ had no significant impact on MDA levels in SH-SY5Y cells. The results showed that RJ treatment 0.3 mg/ml) significantly lowered the activities of SOD and CAT activity while 0.5 mg/ml of RJ had negligible effect indicating that RJ could protect the cell membranes from radical mediated cell injuries.

Key words: Antioxidant, Catalase, Flavonoids, Royal Jelly, SH-SY5Y
1. Introduction

Royal jelly is a yellowish, gelatinous bee product which is secreted by the hypopharyngal and mandibullar glands of worker honeybees and strongly affects the larval growth in the hive that includes the queen bee (Hu et al., 2019; Gismondi et al., 2017). RJ is predominantly composed of water (60% to 70%) carbohydrates (11% to 23%), peptides, proteins (9% to 18%), fatty acids, lipids (4% to 8%), vitamins and mineral salts (Melliou and Chinou, 2005, Ramadan and Al-Ghamdi, 2012; Malka et al., 2009; Fratini et al., 2016). Royal jelly have been shown to have many biological activities that include antibacterial (Ramanathan et al., 2018; Fujiwara et al., 1990), anti-inflammatory (Yanagita et al., 2011), vasodilative and hypotensive (Pan et al., 2019), disinfectant, antioxidant (Park et al., 2019), and antitumor activities. These activities are predominantly credited to its flavonoid content.

Flavonoid has been reported to exhibit a wide range of biological activities, including anti-bacterial, anti-viral, anti-inflammatory, anti-allergic, anticancer and vasodilatory actions (Ayna et al., 2020; Abotaleb et al., 2019; Yin et al., 2019). The flavonoids that include acacetin, apigenin, chrysin, kaempferol, pinocembrin, hesperidin and quercetin contribute to its antioxidant activity (Kocot et al., 2018; Pasupuleti et al., 2017; Viuda-Martos et al., 2008). Flavonoids naturally has reported to have anticancer activities thanks to their potent antioxidant activity in conjunction with their ability to impact cell proliferation and induction of apoptosis as reviewed in (Premratanachai and Chanchao, 2014; Pasupuleti et al., 2017).

Neuroblastoma is the most commonly diagnosed cancer during early years of childhood. Despite being accounted for the improperly high despair and fetality amongst the childhood cancers, neuroblastoma has one of the highest casual and complete regression rates (Maris, 2010). The biological activities of 6 dissimilar RJs on the growth of immortalized murine myoblasts, human prostate cancer and human neuroblastoma have been explored in a different study (Gismondi et al., 2017). However, the antioxidant activities of Bingöl Royal Jelly has yet to be studied on SH-SY5Y neuroblastoma cells. This study was designed to explore the antioxidant activities of Bingöl RJ on SH-SY5Y neuroblastoma cells.

2. Material and Methods

2.1. Cell culture

SH-SY5Y cells were maintained at 37 °C in a humidified 5% CO₂ in Dulbecco's Modified Eagle Medium completed with 10% FBS, 1% penicillin-streptomycin in 25 cm² cell culture flasks for 24 h. Following day, the cells were exposed to different concentrations of RJ between 0.15 and 30 mg/ml for 24 h. The control cell was grown in the same medium without RJ.

2.2. Detection of intracellular reactive oxygen species (ROS) level

Cellular ROS creation was evaluated by use of DCFH-DA assay kit purchased from Abcam, MA, USA, as previously defined (Ayna, 2021, Özbolat and Ayna, 2021). Cells were treated as given in cell culture. Following treatment and growing the cells, 1 × 10⁶ SH-SY5Y cells were collected and incubated for 1 h at 37 °C in the presence of 2 μM DCFH-DA and fluorescence was measured.
2.3. Examination of MDA level

For evaluating malondialdehyde (MDA) level in RJ exposed SH-SY5Y cells, a minor adjustment of previously published protocol was utilised (Ayna, 2020). SH-SY5Y were grown as described in cell culture. The cells were scraped and centrifugation was performed for five minutes at 3000 rpm. Later, the cell suspensions were treated with LPO assay cocktail containing 70% w/v trichloroacetic acid and 1 ml of 0.8% w/v thiobarbituric acid and was incubated for half an hour at 95 °C. Subsequent to that, the mixture was incubated on ice for five minutes and afterwards, the suspension of the cells were centrifuged at 15000 rpm for 10 min. The absorbance measurement of the supernatant was taken at 532 nm.

2.4. Measurement of CAT and SOD activity

The cells were treated as stated in cell culture. The assays were performed following the manufacturer’s instructions provided with the catalase Assay kit (Elabscience), SOD Assay ELISA kit (SunRed).

2.5. Statistical analysis

All of the experimentation procedures were rerun for at least three replicates. The data was statistically analysed and the data groups (comparable) were evaluated by GraphPed Prism 5 by one-way ANOVA Nemwan-Keuls PostHoc Test; p < 0.05 was considered as significant.

3. Results and Discussion

The effects of RJ treatment on ROS generation in SH-SY5Y cells were also examined. The results demonstrated that RJ at 0.3 mg/ml dose remarkably augmented ROS level in SH-SY5Y cells while 0.5 mg/ml of RJ had almost no effect on ROS levels (Fig.1).

![Figure 1. Effects of Bingöl RJ on levels of ROS in SH-SY5Y cells.](image)

To understand whether RJ contributed to the apoptosis of SH-SY5Y, LPO assay was performed by 0.3 and 0.5 mg/mL to detect MDA levels. As shown in Figure 2, the level of MDA in SH-SY5Y cells remarkably
increased in the presence of 0.3 mg/ml RJ while 0.5 mg/ml RJ had no significant impact on MDA levels in SH-SY5Y cells.

Figure 2. Effects of Bingöl RJ on levels of LPO in SH-SY5Y cells.

The antioxidant effects of RJ in neuroblastoma cells were also extensively examined within this study. The results showed that RJ treatment (0.3 mg/ml) significantly lowered the activities of SOD (Fig. 3) and CAT (Fig 4) activity while 0.5 mg/ml of RJ had negligible effect indicating that Bingöl RJ could protect the cell membranes from radical mediated cell injuries.

Figure 3. Effects of Bingöl RJ on the activity of SOD in SH-SY5Y cells.
RJ is a bee product with high protein content is a unique nutrient for the queen honey bee. It leads to a substantial elongation of the lifetime of the queen in comparison to the worker honey bees via anti-inflammatory, -oxidant, -cancer and -microbial properties (Fratini et al., 2016; Liu et al., 2008; Nakajima et al., 2009; Kolayli et al., 2016; Silici, 2019). Therefore, RJ has been extensively used in cosmetic and food industries throughout the world (Hameed et al., 2019; Yeung and Argüelles, 2019). Additionally, in vitro and in vivo studies has shown that RJ decreases cell viability and modulates apoptotic pathway in several kinds of malignant cells and impacts the generation of anti-oxidants and the expression of cancer-associated compounds particularly in treated patients with anti-cancer agents (Miyata and Sakai, 2018). Therefore, RJ is considered to have anti-cancer effects on tumour growing and display protecting roles towards drug-stimulated toxicity. RJ have also been verified to be beneficial for inhibition of side effects, the preservation of the quality of life throughout treatment and the enhancement of diagnosis in cancer patients (Porta et al., 2014). To comprehend the machineries of the valuable impacts of RJ, awareness of the alterations stimulated at the biomolecular levels by RJ with regard to cell proliferation, oxidative damage and other cancer-associated elements is reviewed to be indispensable (Miyata and Sakai, 2018). Thus, in this study, we have examined the anti-oxidant effects of RJ against neuroblastoma cell line SH-SY5Y and mechanisms underlying its effects.

Neuroblastoma, developmental tumour of infants, is the most commonly diagnosed cancer during early years of childhood. Despite being accounted for the improperly high despair and fatality amongst the childhood cancers (accounts for ~13% of all pediatric cancer mortality), neuroblastoma has one of the highest casual and complete regression rates (Maris, 2010; Tartik et al., 2016; Du et al., 2017; Jacquemin et al., 2015).

Supposing that the amount of the ROS created within the cell is excessive to overthrow endogenous anti-oxidant respond, irreversible oxidative injuries to the DNA and RNA, fats, and amino acid (proteins) could result in genetic and/or epi-genetic changes causing a dysregulation in oncogenes and key tumor suppressor
genes. Therefore, oxidative damages lead to alterations in the expression of the genes, proliferation of the cell and programmed cell death and has an important function in progression and initiation of the tumor (Jelic et al., 2021). Oxidative injuries to the lipids result in lipid peroxidation that primarily located in the membranes causing a loss in membrane integrity. Their reactive final substances could then harm other biomolecules. During their exposure to low lipid peroxidation, cellular defense mechanisms resulted in adaption, whereas a high concentration of MDA (a marker of LPO) triggers programmed cell death or activation of necrosis. Amongst several types of aldehydes that could be generated as secondary products during lipid peroxidation, MDA is the most extensively researched (Ayala et al., 2014). For understanding whether the cellular processes of RJ treatment on cell survival can be related to specific induction patterns of ROS and LPO, we demonstrate that RJ resulted in a rise in ROS and LPO levels. The effects of several different types and groups of antioxidants on several different cell lines and animals model have been well investigated to date. These antioxidants have also well studied to understand their roles in the cure of several diseases led by oxidative injuries (Ighodaro et al., 2018). In a study, our colleagues have found that chysin increased lead acetate-induced catalase and superoxide dismutase activities (Bengu et al., 2021). In a different study, another antioxidant molecule, hesperidin, increased NaF induced catalase and superoxide dismutase activities (Caglayan et al., 2021). In our study, the catalytic activities of CAT and SOD were considerably reduced in RJ-exposed neuron cells. Bingöl RJ was also shown to increase levels of MDA and ROS on SH-SY5Y cells indicating that RJ could protect the cell membranes from radical mediated cell injuries. All of these findings reveal antioxidant properties of Bingöl RJ.

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Conflict of interest

The author declares that there is no conflict of interests

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