In Vitro Antioxidant Effect of PROPOLIS on VERO Cell Culture.

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Abstract

Propolis is a hive product containing chiefly beeswax and plant-derived substances and volatile substances. Propolis has been used for its antioxidant effect and other actions. The present work was planned to investigate the antioxidant effect of propolis on VERO cell culture exposed either to H_2O_2 for two hours or to serum deprivation for forty eight hours. The obtained results revealed that, propolis can protect VERO cell culture from cell deformation or growth reduction in vitro, where it significantly inhibited cell toxicity induced by either two hrs exposure H_2O_2 or 48 hrs serum deprivations. This result could be reclaimed to inhibition of the oxidative stress and scavenged free radicals by propolis in cell culture. So it could be concluded that, addition of propolis maintain the viability and enhance the growth of VERO cell culture.

Introduction

Propolis is a complex resinous bee product with a variable physical appearance that depends on many factors with different colors where it may be cream, yellow, green, light or dark brown. .Propolis consists of exudates from plants mixed with bees wax and used by bees for diverse purpose, among which as glue in general purpose as sealer and draught excluder for bee hives. Propolis has been long used in folk medicine of different nations as early in Egypt as 3000 B.c (4) Propolis (honey bee glue) is currently used as a healthy food and for the treatment of various ailments. Indeed, it has been shown to have a wide range of biological activities principally attributable to the presence of flavonoids; major component, rutin, quercetin, galangin, etc. (6; 7).Hence, the putative therapeutic properties of propolis could be related to its antibacterial (3), anti-inflammatory (11) and anti-oxidatives (8; 15) activities .More than 200 propolis constituents have been identified by gas chromatography-mass Spectrometry (GC-MG). These compounds can be grouped as follow: free aromatic acids, flavonoids, benzyl, methyl butenyl, phenyl ethyl, cinnamyl and other esters (1: 10). These acids include chalcones and dihydrochalcones, terpenoids and other as sugars, ketons and alcohols (2; 9). The present study aims to raise new data about the antioxidant activity of propolis collected from two different localities in West-Libya, as an enhancing factor for growth of VERO cell culture and its role in improving performance of the living cell.

Materials and Methods

1-Propolis:

Propolis samples were collected from Libya during spring, 2007 from two different localities in Western-Libya. Approximately, 100gm of the collected propolis were directly obtained from the bee hives. The specimens of propolis were stored immediately in ice box at 4^0c for one week and then dehydrated further under vacuum. The dried propolis ground to fine powder. Water extract of propolis (WEP) was prepared using two grams of the specimens were mixed with 25ml of deionized water and the water level marked on the tubes, then shaken at 95°c for 2 hrs and cooling to room temperature. Water was added to the marked level and the contents centrifuged to obtain the supernatant **(13).**

2-Tissue culture:

African Green Monkey Kidney (VERO) cells established by Yasumura and Kawatika (21) were employed in this study. The cells were seeded at density of 2×10^4 per well into 24-well Microtiter plate. Three plates were prepared and used for the present experiment. VERO cells were left for establishment of confluent sheet for 72 hrs prior any treatment.

3-Cell culture media:

Minimum essential medium (MEM), produced by Gibco with Hank's salts, L-glutamine and without sodium bicarbonate was used for propagation and maintenance of VERO cells. It was prepared according to the manufacture's instructions. The medium was supplemented with 10% Fetal Bovine serum (FBS), as growth medium at PH 7.2, and was supplemented with 2-3% FBS for maintenance of the cell culture at the same PH.

4-Fetal Bovine Serum (FBS):

Fetal bovine serum which (virus and mycoplasma screened) was used as supplement for cell culture media. It was supplied by Gibco- USA.

5-Experimental design:

After establishment of confluent sheets, VERO cells in the three tissue culture plates were divided into 6 groups. Each group was as a raw of 4 successive wells. Group 1 was kept as control without any treatment, group 2 was exposed to H_2O_2 (0.2mM PH 3.0) for 2 hrs, group 3 exposed to serum deprivation for 48hrs (by addition of only 0.1% FBS to the supplemented MEM, group 4 was exposed to propolis extract, group 5 was exposed to H_2O_2 as previously mentioned with propolis extract while the last group (6) was exposed to the previously mentioned serum deprivation with addition of propolis. Different concentrations of propolis were used in this experiment as 40, 4; 0.4ug/ml in plate-I, II and III respectively. After the end of each treatment exposure, the cells were counted in each well using a haemocytometer.

6-Statistical analysis:

Data are presented as mean ±SEM. Statistical comparisons were made by means of a one-or two-way analysis of variance (ANOVA) followed by Student's t-test **(18).** $P< 0.05$ was considered statistically significant.

Results and Discussion

Table (1): Schedule of VERO cell culture treatment

Cell culture group	Applied treatment
1	No treatment
2	Exposure to 0.2mM H_2O_2 at pH 3for 2 hours
3	Exposure to serum deprivation for 48 hours (only 0.1% FBS was added to MEM)
4	Addition of propolis at a concentration of 40, 4 and 0.4mg/ml in three plates respectively
5	Exposure to 0.2mM H_2O_2 at pH 3 with addition of propolis extract 40, 4 and 0.4mg/ml in three plates respectively
6	Exposure to serum deprivation for 48 hours with addition of propolis extract 40, 4 and 0.4mg/ml in three plates respectively

Table (2): Cell count of treated VERO cells in different groups

Cell culture group	Cell count (10^4/ml)		
	Plate-1♦	Plate-2♦♦	Plate-3♦♦♦
1	8.7	9.3	8.9
2	6.7	7.0	7.1
3	3.4	3.2	3.3
4	9.4	9.7	9.1
5	7.5	7.6	7.2
6	4.4	4.6	4.1

♦ Plate-I= addition of 40mg propolis/ml
♦♦ Plate-II= addition of 4mg propolis/ml
♦♦♦Plate-III= addition of 0.4mg propolis/ml

The present work was conducted to investigate the effect of propolis in vitro using VERO cell lines. The experimental design depended on studying the cell viability after exposure to H_2O_2 or serum deprivation with the addition of propolis.It was found that addition of propolis to cell cultures that exposed either to H_2O_2 addition in their media or to serum deprivation markedly improved the cell viability. This result was noticed after performing cell count using haemocytometer which revealed that the number of control cells (group-1) was ranged between 8.7×10^4 up to 9.3×10^4 cells / ml and cell count of those exposed to H_2O_2 (group-2) was decreased to be ranged between 6.7×10^4 and 7.1×10^4

cells / ml with a sharp decrease in the cell count of cells exposed to serum deprivation (group-3) which was ranged between 3.2×10^4 and 3.4×10^4/ml. On the other side it was noticed that addition of propolis extract to the cells with the previously mentioned two exposures improved the cell vitality and viability and the damage were markedly corrected in group-5 that exposed to H_2O_2+ propolis.

On the other hand, addition of propolis to the cells of group-6 wells (that exposed to serum deprivation + propolis addition) revealed only weak improvement in cell viability as comparing with the previous group. Moreover, addition of propolis extract alone to the cell culture improved the cell viability when compared with the cells in the control wells. The last mentioned improvement in cell viability in groups-5 and 6 was propolis concentration related, as the cell viability increased with the increased propolis extract concentration as presented in table (2).These findings showed that propolis was a more potent inhibitor of induced cell death after being exposed to H_2O_2 or serum deprivation. Addition of H_2O_2 to cell culture induced cell toxicity in the three plates by 23, 24.7 and 20.2% respectively as compared with the control (group-1).

It is well known that reactive oxygen species (ROS) such as H_2O_2, nitric oxide, superoxid anion and hydroxyl radical, have been implicated in the regulation of many important cellular events including transcription factor activation and cellular proliferation (16: 12). However, excessive production of ROS gives rise to events that lead to death in several types of cells (20). In addition, It has been reported (19) that ROS have induced death in vitro in their study on cultured neurons and cultured PC12 cells.Concerning the effect of serum deprivation on VERO cells.

It was found that deleterious effect which could be pointed to the depletion of certain important elements present in the serum that needed for the growth and viability of VERO cells. This opinion was supported by a previous study (17) where the depletion of glutamine and serine and the metabolic deviations leading to a shortage of intermediate products required for other metabolic pathways probably contribute to the lower cell yield and higher cell death rate in serum free media.

Also, It was stated (14) that complete removal of serum for a period of 3 hours resulted in a 3-hour prolongation of the cell cycle regardless of the time after mitotic selection at which the serum was removed. For synchronized cells, the rate of entry into both the S phase and into the subsequent cell cycle were reduced in 3% serum as compared to 30% serum, the former rate being significantly greater than the latter at both serum concentrations. Our results thus indicate that these cells are continuously dependent upon serum throughout the entire cell cycle.

It was clear that propolis inhibited cell death in VERO cell cultures exposed to either H_2O_2 or to serum deprivation and such inhibition of cell death was propolis concentration related as 40ug/ml propolis caused more inhibition of cell death and increased cell vitality than the other two concentrations (4 and 0.4ug/ml). This could be attributed to the fact that propolis acts against oxidative stress, lipid peroxidation and exhibited a free radical scavenging action. This opinion agreed with some previous studies

(4: 5) where propolis exhibits strong scavenging activity in vitro towards both the superoxoid anion radical and the nitrous oxide radical..

So, it could be concluded that propolis is a VERO cell protective acted as an antioxidant against lipid peroxidation and free radical production with an action degree depends on its concentration.

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تأثير البروبيوليز على خلايا الزرع النسيجي لكلى القرد الأخضر الأفريقي

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القاهرة-العباسية-ص٠ب٠ ١٣١

تنتج مادة البروبيوليز بواسطة النحل لأغراض متعددة داخل الخلية وهى مادة ذات تأثير مضاد للأكسدة٠ وقد أوضحت نتائج الدراسة الحالية هذا التأثير على خلايا كلى القرد الأخضر الأفريقي المستمرة حيث تغلبت مادة البروبيوليز على التأثير المؤكسد لماء الأكسيجين وكذلك التأثير السام نتيجة حرمان الخلايا من المصل المضاف إلى الوسط الغذائي وقد زاد البروبيوليز من الحيوية وعدد الخلايا في المجموعات المختلفة حيث أنه له القدرة على تقليل مادة فرى راديكال٠كذلك لوحظ أن إضافة هذه المادة إلى الوسط الغذائي للخلايا يساعد على تقليل نسبة المصل المستخدم٠