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RESEARCH ARTICLE

Evaluation of effects on hepatocellular carcinoma cell line of *Cocos nucifera*: In vitro study

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Objective: Cancer is one of the most important diseases today. The use of chemical drugs, surgical operations, and transplants is very common in its treatment. In addition to these treatment methods, studies include the effects of natural and plant-derived substances. Various substances are used in these studies, which are called phytotherapy. The antioxidant activity and cytotoxic effects of Cocos nucifera on hepatocellular carcinoma HepG-2 and the mouse fibroblast L929 cell line were investigated in this study. **Methods**: In this study, in vitro cytotoxic effects of C. nucifera at different concentrations (7.81-500 mg/ml) were investigated on the L929 Mouse Fibroblast cell line and HepG-2 Hepatocellular Carcinoma cell line. In addition to these studies, their antioxidant capacity was evaluated via spectrophotometric methods. In this work, different concentrations of C. nucifera were examined. **Results**: According to the results, C. nucifera had a cytotoxic effect in HepG-2 and ensured the proliferative effect of cells in the L929 cell line. Among C. nucifera extracts according to total antioxidant capacity results, C. nucifera extract was found to be the richest in antioxidants with 2.79 mmol/L, while the material with the lowest antioxidant capacity was determined to be C. nucifera milk. DPPH free radical scavenging activity results show the opposite. **Conclusion**: In line with the data we obtained, it is thought that C. nucifera can be used in liver cancer studies, and its antioxidant effect may play an important role in balancing against oxidative stress. Simultaneously, the data show that the exposure time and concentrations of the active substance are related to the cytotoxic effect. However, it may be considered that the use of C. nucifera water, extract, and milk in cancer patients may be supported by more comprehensive clinical studies.

Keywords: Cocos nucifera, HepG-2, L929, cytotoxicity, antioxidant activity

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Introduction

Today, plants maintain their place in the cosmetics, health, pharmaceutical, textile, and various industrial fields. Its use in the health and pharmaceutical industries started in ancient times, and its history dates to the first ages [1,2,3]. Recently, the production of plant-derived drugs has been increasing in developing countries in the fields of medicine and biotechnology [3]. The use of plants in treatments over time is called "Phytotherapy" [2]. Phytotherapy is also used for treating cancer, which is one of the most important diseases today. The coconut (*Cocos nucifera*) plant, which we have chosen as a phytotherapy agent, has been used in many cancer studies [4,5]. Milk obtained from *Cocos nucifera* fibers may be a source of new drugs with antitumor and anti-multidrug resistance activities. They tested different forms of *C. nucifera* milk on the human erythroleukemia cell lines K562 and Lucena-1. They wrote that *C. nucifera* milk reduced multiple resistance and cell viability by half in the Lucena-1 cell line [4]. The anticancer effect of tripalmitin and trilaurine glycerides in *C. nucifera* oil is known. Antioxidant-rich polyphenol compounds in *C. nucifera* oil have anti-inflammatory and antioxidant effects in colorectal cancers, and provide improvements, thus having an anticancer effect [6].

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Coconut (*Cocos nucifera*), which belongs to the *Arecaceae* family, is the fruit of the tropics. It has a supportive, strengthening effect on the defense and intestinal systems and is very nutritious. *C. nucifera* is a product of the tropics. *C. nucifera* dates grow on sandy soils and are highly tolerant of soils with high salt content. It prefers regions with plenty of sunlight and regular precipitation [7]. *C. nucifera* is a large palm that reaches up to 30 meters in length. *C. nucifera* is a hard-shelled fruit [8]. *C. nucifera* development progresses from leafy to fruition. It is an endocarp in a third layer other than the exocarp and mesocarp that make up the shell [8,9]. *C. nucifera* is very rich in protein, fat, carbohydrates, and fiber. It contains pantothenic acid, pyridoxine, riboflavin, niacin, and thiamin, as well as vitamins C, E, and K. *C. nucifera,* which is rich in minerals, is first given sodium and potassium; it contains elements such as iron, calcium, copper, phosphorus, selenium, zinc, magnesium, and manganese. Additionally, many other nutritional components beneficial for health are also included in its structure [10].

The clear, sweet, and slightly acidic liquid in the interior of a *C. nucifera* (in the endocarp section) is referred to as *"Cocos nucifera* juice." This is considered by many to be the "perfect drink" for its refreshing nature and naturalness. *C. nucifera* water is sterile and has not been hit or injured. It is a ready-made source of clean drinking water in any possible case. Its sodium and potassium content make

it an ideal drink for rehydration. Owing to the nutritional properties of *the* ingredients contained in *C. nucifera* water, it has antioxidant, hepatoprotective, antifungal, antimicrobial, antiviral, and anticancer effects [11,14]. *C. nucifera* milk is an opaque, milk-white liquid derived from the grated meat of mature *C. nucifera.* The efficiency and quality of *C. nucifera* milk from *C. nucifera* meat depends on the temperature of the added water the water ratio [15]. *C. nucifera* milk is an emulsion that mainly contains lipid, carbohydrates, and proteins. *C. nucifera* oil is a type of oil derived from the seed, succulent tissues, and milk of the plant [12]. Its content is quite rich, and its use is very common. This study determined the antioxidant activity and *in vitro* cytotoxic effects on Hepatocellular Carcinoma (HepG-2) cells and the Mouse Fibroblast (L929) cell line of *C. nucifera*.

Methods

Preparation of C. nucifera

Four *C. nucifera* were used the study. A hole was made in each *C. nucifera* and the inner water was poured into a sterile container. *C. nucifera*, whose juice was filtered, was divided into two parts, and the fleshy part was separated from the shell part. *C. nucifera* milk was obtained from these fragments (Figure 1).

Cell Culture Procedure

Hepatocellular Carcinoma (HepG-2) cells and Mouse Fibroblast (L929) cells were used in my study. Cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 20µg/ml penicillin/streptomycin (100 UI), and 1% L-glutamine at 37°C in 5% CO_2.

MTT Colorimetric Test

The cytotoxic activity was assessed using 3-(4,5 dimethyltiyazole 2-il)-2.5 diphenyltetrazilium bromide (MTT) colorimetric analysis. For this purpose, 96 wells plates were used and the experiment was carried out in a total volume of 200 µL. $5x10^3$ cells were cultured in each well of the 96 plates. DMEM and cells were used as positive controls, while only DMEM was used as a negative control. *C. nucifera* inner water, extract and milk concentrations were added as 500-250-125-62.5-31.25-15.62-7.81 mg/ml, and incubated for 24 and 48 h. The liquid in the incubation wells was aspirated (50 µL /well), followed by the cells' processing for 3 h at 37°C with MTT (Sigma Aldrich) solution (10 µL 5 mg/mL PBS-phosphate-buffered saline). Finally, the cells were broken down with 100 µL DMSO (Dimethyl sulfoxide). Absorbance was measured at 570 nm using an ELISA (Enzyme-Linked ImmunoSorbent Assay) microplate reader. The percentage of living cells determined by the equation % viability = (absorbing processed cells/absorbing control cells) ×100 was determined. IC_{50} cell growth, the sample amount that provides 50% inhibition, was calculated from a dose-response curve. The cytotoxic effect of *C. nucifera* internal water, extracts, and controls was evaluated by comparing the IC_{50} values of samples for all cell lines.

Determination of Antioxidant Activity

Total antioxidant capacity measurement

The ELISA method was used to determine the antioxidant capacity of the samples. The data were determined by a spectrophotometer reading at 600 nm.

DPPH (1,1-diphenyl 2-picril hydrazil) free radical sweeper activity determination

DPPH• free radical removal activity was carried out according to a variant of the Blois method (1958). DPPH (1.1-Diphenyl-2-picrylhydrazyl radical) was calculated and weighed as 0.1 mM. The weighed amount was dissolved in ethanol and stored at +4 °C in the dark. It was

Fig. 1. Preparation of ***Cocos nucifera***

applied to samples at twice the rate, and the sample was incubated in the dark for 30 min at 37°C and measured at 517 nm. After the first measurement, it was incubated in the dark at 37° C for another 30 min and measured at 517 nm (Figure 2).

Statistical analysis

In the study, the data was presented as a mean and standard deviation. The cell analysis data were calculated with the Excel (2016) program and assigned statistical importance. The IC_{50} and EC_{50} (half the highest inhibitory concentration-half the maximally effective concentration) values were determined with the GraphPad Prism (9.0) program.

Results and discussion

C. nucifera oil was found to reduce liver damage, regenerate hepatocytes, increase apoptosis, and, most importantly, reduce cancerous cells in studies on mice with liver damage and hepato-steatosis [16,17]. In studies like these, many types of cancer have been studied. The oral cancer cell line KB and liver cell line HepG-2 emphasized that the effect was not uniform and varied in the study treatment with different concentrations of pure *C. nucifera* oil, processed *C. nucifera* oil and fractionated *C. nucifera* oil for 72 h. It is stated in the literature that all oil types have suppressive effects against two types of cancer, albeit at different rates and percentages [18]. The cytotoxic effects and antioxidant activity of *C. nucifera* at various concentrations on HepG-2 were investigated in cell culture studies, but L929 was not as healthy as the control. The antioxidant activity and cytotoxic effects on HepG-2 cells and the L929 cell line of *C. nucifera* were determined in our study.

In L929 cells, the best viability percentage for *C. nucifera* milk was determined at 250 mg/ml within 24 and 48 h. The concentration of *C. nucifera* internal water was 31.25 mg/ml after a 24 h treatment and 500 mg/ml after a 48 h treatment.. The best viability percentage was determined at 125 mg/ml in 48 h and 500 mg/ml in 24 h (Figure 3).

When the proliferation effect of *C. nucifera* in the L929 cell line is examined according to the average viability, it varies according to the water and milk it contains. We observed that the concentration of the active substance applied to the cells changed with time. The highest vitality was determined at the 24th hour with 191.37%, while the lowest was determined at the 48th hour with 77.12%.

It was discovered that the essence of *C. nucifera* on HepG-2 hepatocellular carcinoma cells produced the best results of 250 mg/mL in 24 and 48 h. The best viability of the inland water effect on cells was determined at 7.81 mg/ml in 24 h and 62.5 mg/ml in 48 h. The percentage of the vitality of *C. nucifera* milk in cells was 15.62 mg/ml and 250 mg/ml respectively, in 24 and 48 h (Figure 4).

Three versions of *C. nucifera* were used in the experiments, and according to the results of the 24-hour application on the HepG-2 cell line, known as the cancerous cell line, the average order of their vitality was CNS_{48} > CNS_{24} > CNs_{48} > CNS_{24} > CNS_{24} >$CNÖ_{48,}$ the cytotoxicity ranking in IC_{50} values is CNS_{24} > CNS_{24} > CNS_{48} > CNS_{48} > CNS_{24} > CN $milk_{48}$ (Table 1).

The *C. nucifera* extract used in this study has a high oil content, and although it varies depending on the concentration difference in the HepG-2 cell line, like the other studies, significant results were found in terms of effect. *C. nucifera* juice has been evaluated in various liver cancer studies. In some studies, it cannot directly prevent liver cancer but can reduce the damage [19]. It has been determined that it delays the progression of breast cancer by inducing apoptosis, suppressing metastasis, and activating antitumor immunity in the 4T1 breast cancer cell line. This study was conducted considering that, some concentrations showed an effect [20]. The effect of *C. nucifera* milk on cancer has not been evaluated in studies. And various concentrations of *C. nucifera* milk were applied to the HepG-2 hepatocellular carcinoma cell line, which was prepared in a concentrated form with water, and to the mouse fibroblast cell line, L929, which was determined to be the control. As a result, after the application, it was observed that *C. nucifera* milk and extract could be used as a good activator in L929 cells, while it was also observed that it supported growth in HepG-2 hepatocellular carcinoma cells at some doses and suppressed it at others.

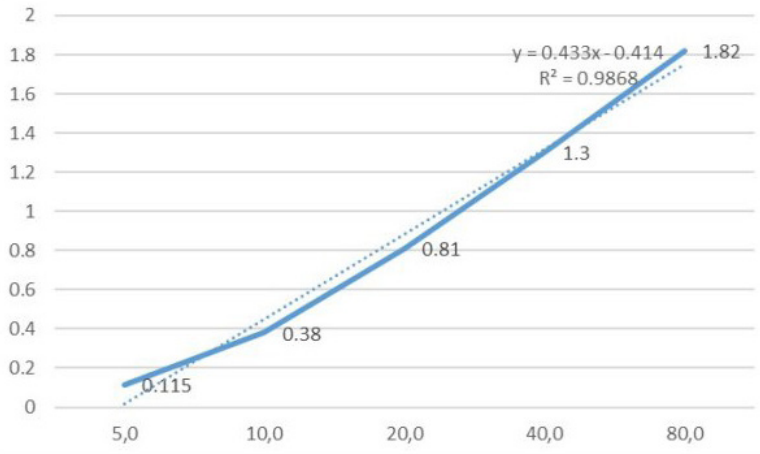

Fig. 2. DPPH standard graphic

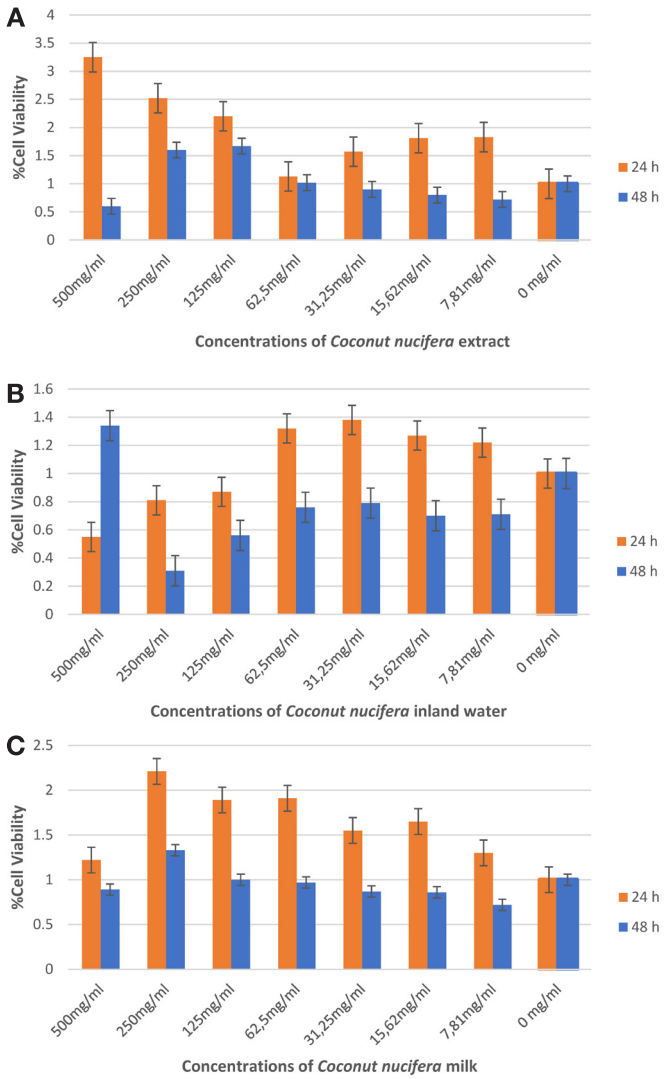

Fig. 3. % Viability results in L929 Mouse Fibroblast cells of A. Extract of ***Cocos nucifera***; B. inland water of ***Cocos nucifera***; C. milk of ***Cocos nucifera***

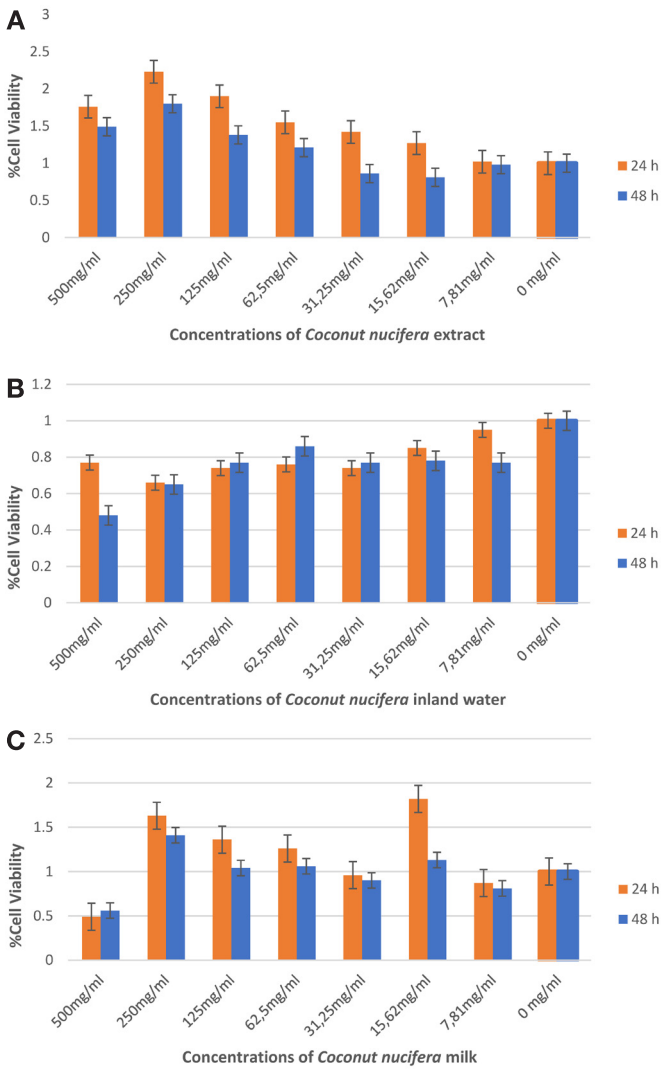

Fig. 4. Results of % viability in HepG-2 hepatocellular carcinoma cells of A. ***Cocos nucifera*** extract; B. ***Cocos nucifera*** juice; C. ***Cocos nucifera*** milk.

Table 1. ***C. nucifera*** effect on cytotoxic Hepatocellular Carcinoma HepG-2 cells

	Extract		Inner water		Milk	
	24 h	**48 h**	**24**	**48**	**24**	**48**
IC_{50}±SD (µg/ml)	63.49±8.21	93.13±9.53	93.13±19.53	96.37±33.55	16.35±17.71	71.96±19.70

Table 2. The effect of ***C. nucifera*** on mouse fibroblast L929 cells

	Extract		Inner water		Milk	
	24 h	**48 h**	**24**	**48**	**24**	**48**
IC_{50}±SD (µg/ml)	99.6±10.7	137.2±12.0	39.0±19.4	97.8±12.6	132.6±14.38	7.8±5.6

Table 3. Antioxidant results and evaluation of ***C. nucifer*** (extract, inner water, and milk)

Examples	mmol/L	assessment
C. nucifera extract	2.79	Very good
C. nucifera milk	0.94	The very low antioxidant level
C. nucifera inner water	1.91	normal

The results of the substances that were made and measured according to the procedure of the total antioxidant capacity measurement kit (Rel Assay Diagnostic, Turkiye), were interpreted based on the procedural reference values. The antioxidant effects of *C. nucifera* were found in water, extract, and milk. Their levels were evaluated (Table 3). The results are consistent, considering the studies.

The DPPH activity results of the samples were obtained and compared with the antioxidant BHT (Butyl Hydroxy Toluene) and evaluated using the standards. It was found that they have antioxidant values of *C. nucifera* extract 21.40 mg/ml, *C. nucifera* internal water 22.04 mg/ml, and *C. nucifera* milk 22.14 mg/ml. According to the comparison between them, the best activity was detected in *C.*

nucifera milk, while the lowest activity was found in *C. nucifera* extract. Supporting the antioxidant data in the study, Nevin and Rajamohan in their 2004 study determined that pure *C. nucifera* oil decreased total cholesterol, triglycerides, phospholipids, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and increased high-density lipoprotein (HDL) cholesterol levels [12]. Additionally, many studies support these results [21, 22].

Conclusion

In line with the data we obtained, it is thought that *C. nucifera* can be used in liver cancer studies, and its antioxidant effect may play an important role in balancing against oxidative stress. Simultaneously, the data show that the exposure time and concentrations of the active substance are related to the cytotoxic effect. However, it may be considered that the use of *C. nucifera* water, extract, and milk in cancer patients may be supported by more comprehensive clinical studies.

Author contributions

GAA - Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft.

ZT - Conceptualization, Data curation, Methodology, Visualization, Investigation.

EA - Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Writing – original draft.

Conflict of interest

None to declare.

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