The antioxidant activity of curcumin extract against HepG2 cells

Abeer A Khamis (1), Amira H Sharshar* (1), Amal H Mahmoud (2), Tarek M Mohamed (1)

(1) Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University
(2) Head Researcher of Special Food and Nutrition Department, Food Technology Research Institute, Agricultural Research Center, Giza

Correspondence:
Amira H Sharshar
Researcher Assistant at Food and Nutrition Department
Food Technology Research Institute
Agricultural Research Center
Amirahassan29@yahoo.com
Tel: +201006135205

Abstract

Oxidative stress plays a major role in the pathogenesis of various diseases including neurodegenerative diseases, myocardial ischemia-reperfusion injury and cancer. The aim of this work was to extract curcumin from curcuma longa then evaluated its antioxidant activity against HepG2 cells. FTIR was used to confirm the structure of the extracted curcumin. Antioxidant activity was assessed by DPPH method. Results showed that curcumin had antioxidant activity with Ic50 value of 3.7±0.20 μg/ml. Moreover treatment of HepG 2 cells with curcumin resulted in decreasing the concentration of L-malondialdehyde (L-MDA) and increasing the activity of the antioxidant enzymes superoxide ismutase (SOD) and catalase. In conclusion, the present study showed the effect of curcumin as a natural antioxidant compound that enhanced the antioxidant defense system in HepG2 cells by increasing the activity levels of antioxidant enzymes SOD and catalase and decreasing the concentration of L-MDA.

Key words: Antioxidant activity, curcumin, catalase, superoxide dismutase

1. Introduction

Oxidative stress occurs in cells and tissues of organisms wherever oxidation and reduction processes abound. This has been attributed to production of reactive oxygen species (ROS) beyond the ability of the system to neutralize them (Halliwell, 1996). Oxidative stress has been incriminated in the pathogenesis of several disease
conditions such as cancer, cardiovascular, liver injury, myocardial infarction, Alzheimer’s disease, Parkinsonism as well as in the ageing process (Grzegorczyk et al., 2007).

Continuous exposure to synthetic compounds, which is characteristic of orthodox medicine, could lead to increase in the amount of free radicals in the body and hence cause different tissue and/or biochemical lesions. A lot of scientific researches has focused on screening plants and herbs for their potential antioxidant properties because active principles from these plants are thought to play important roles in preventing diseases caused by oxidative stress (Bulus et al., 2017).

Curcumin is extracted from Curcuma longa (turmeric) rhizomes is the main ingredient in turmeric and curry (Tayyem et al., 2006). Turmeric was used in China and India many years ago as a traditional medicine (Teiten et al., 2010). Several studies have shown that curcumin exerts antioxidant, anti-inflammatory, anti-carcinogenic and chemopreventive activities on many tumor cells.

The essential structure of curcumin is the feruloylmethane skeleton. It consists of two benzene rings, one having a phenol hydroxy and the other having a phenol methoxy showing the ability of curcumin to act as a hydrogen donor eliminating oxygen free radicals (Lv et al., 2016). Curcumin influences cell death by multiple, interdependent processes. It exerts its anticancer and antioxidant activity by modulating the expression level of several target genes. (Chiu and Su, 2009; Choudhuri et al., 2002; Perrone et al., 2015).

2. Materials and methods

2.1. Extraction of curcumin from Curcuma Longa

Extraction of curcumin was carried out according to the method explained by (Kulkarni et al., 2012) using soxhlet extraction followed by column chromatography.

2.2. Fourier transforms infrared spectroscopy

A JNS-CO Spectrum System 4100 LE FTIR spectrometer (Japan) was used for the analysis of purified extract of Curcuma Longa. The spectral FTIR are measured at mid infrared region corresponding to wavenumbers of 4000-650 cm⁻¹ with resolution of 4 cm⁻¹. (Lestari et al., 2017)

2.3. Determination of antioxidant activities of curcumin
Total antioxidant activity was estimated according to the free radical diphenylpicrylhydrazyl (DPPH) method described by (Molyneux, 2004).

2.4. Cell line and cell culture
HepG2 cells (ATCC, Manassas, Virginia, USA; Cat.no. HB 8065) were cultured in DMEM medium (GIBCO, New York, USA; Cat.no.11995073) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, New York, USA; Cat.no.10099133), 1% penicillin/streptomycin (Thermo Fisher Scientific; Waltham, MA, USA; Cat.no. SV30082) and 2% L-glutamine (Invitrogen, Grand Island, New York, USA; Cat.no. 25030024) in a humidified atmosphere of 95% air with 5% CO₂ at 37°C (Jiang et al., 2013a).

2.5. Determination of antioxidant activity of curcumin in HepG-2 cells:
L-Malondialdehyde (L-MDA) was determined according to the method of (Macotpet et al., 2013). The activity of superoxide dismutase (SOD) was determined according to the method of (Weydert and Cullen, 2010). One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute. SOD Activity: IU/ml = % inhibition x 3.75. Catalase activity was determined according to the method described by (Aebi, 1984).

3. Results
3.1. Determination of curcumin in Curcuma Longa
Curcumin was extracted from Curcuma Longa as described previously. The identity of isolated curcumin was further confirmed by FTIR (Fig.1).

FTIR spectrum of curcumin showed a characteristic stretching band of O-H at 3512 cm⁻¹. The peak at 3014 cm⁻¹ represents C-H Streching and 1602 cm⁻¹ peak was assigned to C=C symetric aromatic ring streching. The peak at 1506 cm⁻¹ represents C=O, while enol C-O peak was obtained at 1280 cm⁻¹ and benzoate trans-C-H vibration was at 962 cm⁻¹.
3.2. Antioxidant activity of curcumin

By using DPPH free radical scavenging activity the antioxidant activity of the curcumin was evaluated by comparing the IC<sub>50</sub> of the ascorbic acid. The IC<sub>50</sub> of the curcumin was found to be 3.7±0.20 μg/ml which is closer to the ascorbic acid 2.2±0.23 μg/ml (Fig. 2).
3.3. Assessment of antioxidant effect of curcumin

Our results revealed a significant (P≤0.05) decrease in the level of L-MDA in HepG2 cells of control groups (G1) (Fig. 3). This reduced level was significantly (P≤0.05) elevated after administration of standard curcumin at a dose of 10 ug/ml when applied for 24h (G2). Similarly, the extracted curcumin (at dose of 10 and 20 ug/ml) resulted in a significant increase in L-MDA concentration when applied for 24h (G3 and G4) as compared to control group. In general, the standard curcumin showed a significant higher L-MDA concentration than that of the extracted curcumin with no significant difference in the L-MDA concentration was shown between treatment by low dose (10 ug/ml) and high dose (20 ug/ml).

In case of detecting SOD activity, our results revealed a significant (P≤0.05) decrease in the level of SOD in HepG2 cells of control groups (G1) (Fig. 4). This reduced level was significantly (P≤0.05) elevated after administration of standard curcumin at a dose of 10 ug/ml when applied for 24h (G2). Similarly, the extracted curcumin (at dose of 10 and 20 ug/ml) resulted in a significant increase in SOD level when applied for 24h (G3 and G4) as compared to control group. In general, the standard curcumin showed a significant higher levels of SOD than that of the extracted curcumin with no significant difference in the SOD concentration was shown between treatment by low dose (10 ug/ml) and high dose (20 ug/ml).
Also our results revealed a significant (P≤0.05) decrease in the level of CAT in HepG2 cells of control groups (G1) (Fig. 5). This reduced level was significantly (P≤0.05) elevated after administration of standard curcumin at a dose of 10 ug/ml when applied for 24h (G2). Similarly, the extracted curcumin (at dose of 10 and 20 ug/ml) resulted in a significant increase in CAT level when applied for 24h (G3 and G4) as compared to control group. In general, the standard curcumin showed a significant higher CAT level than that of the extracted curcumin with no significant difference was shown between treatment by low dose (10 ug/ml) and high dose (20 ug/ml).

**Fig. (3):** Effect of curcumin on L-MDA concentration in HepG2 cells, Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).
Fig. (4): Effect of curcumin on SOD activity in HepG2 cells, Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

![SOD Activity Graph](image1)

Fig. (5): Effect of curcumin on catalase activity in HepG2 cells, Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

![Catalase Activity Graph](image2)
4. Discussion

In recent few years, several natural products extracted from medicinal plants have been studied for their antioxidant and anti-cancer effects by inhibiting the proliferation, invasion, and metastasis of cancer cells. However, several types of research have revealed the mechanisms of their action, much research is still needed. (Chang et al., 2017) Curcumin which is extracted from the rhizomes of Curcuma longa sp. has received attention as a potential agent in cancer therapy. (Shariati et al., 2017) Accumulating evidence shows that curcumin has a diverse range of molecular targets, and therefore acts upon numerous molecular and biochemical cascades. (Gallardo and Calaf, 2016)

The aim of the present study was to extract curcumin from curcuma longa and evaluate the antioxidant activity of curcumin against human cancer in vitro.

In the present study curcumin was extracted from curcuma longa and the structure of the extracted curcumin was confirmed by using FTIR

The FTIR spectrum revealed the presence of stretching band of O-H at 3512 cm\(^{-1}\), C-H Stretching band at 3014 cm\(^{-1}\), a peak at 1602 cm\(^{-1}\) peak which was assigned to C=C symmetric aromatic ring stretching, a peak at 1506 cm\(^{-1}\) represents C=O, while enol C-O peak was obtained at 1280 cm\(^{-1}\) and benzoate trans-C-H vibration was at 962 cm\(^{-1}\). This FTIR spectrum of the extracted curcumin was matching with the FTIR spectrum of the standard curcumin. Also some studies use FTIR spectroscopy for identification of curcumin isolated from different curcuma species for instance, (Lestari et al., 2017) performed FTIR spectroscopy to determine the curcuminoid content isolated from curcuma xanthorrhiza.

In this study, the antioxidant activity of curcumin was evaluated using DPPH scavenging assay. 50% of inhibitory concentration (IC 50) value was 3.7±0.20 μg/ml. The curcumin structure contains a variety of functional groups including the β-diketo group, carbon–carbon double bonds and phenyl rings containing varying amounts of hydroxyl and methoxy substituents which may be responsible for its antioxidant activity against DPPH (Wright, 2002).

Oxidative stress is defined as an excess of reactive oxygen species (ROS) due to imbalance between the rate of ROS production and ROS removal. Overproduction
of free radicals and reactive oxygen species (ROS) interact with important biological molecules such as DNA, lipid or protein leading to many degenerative diseases, such as cancer. The antioxidant activity of curcumin makes it able to eliminate oxygen free radicals serving as a hydrogen donor or adjust other mediators involved in the oxidation reaction against antioxidant and anti-lipid peroxidation (Lv et al., 2016). Since oxidative stress triggers various antioxidant mechanisms in the body biomarkers such as lipid peroxidation products and endogenous enzymes with antioxidant properties have been used to assess oxidative stress (Trujillo et al., 2013).

Malondialdehyde (MDA) one of the end products of lipid peroxidation has been widely used as a biomarker of oxidative stress (Macotpetl et al., 2013). In this work L-MDA was used to monitor the oxidative stress status in curcumin treated HepG 2 cells. The results obtained in this work demonstrated that curcumin decreased the concentration of L-MDA in HepG 2 cells. The result of (Yeni et al., 2017) also showed that curcumin decreased MDA level in preeclampsia-induced cell.

Superoxide dismutases (SODs) are essential enzymes that catalyze the conversion of superoxide into oxygen and hydrogen peroxide. Through their activity, SOD enzymes control the levels of a variety of reactive oxygen species (ROS) and reactive nitrogen species, thus both limiting the potential toxicity of these molecules and controlling broad aspects of cellular life that are regulated by their signaling functions (Wang et al., 2018). This study showed that curcumin treated cells showed increased activity of SOD than untreated cells. This result is similar to the results of (Aziza et al., 2014) that showed that curcumin treatment enhanced the activity of SOD in colon cancer. Also the results of (Jagetia and Rajanikant, 2015) indicated that curcumin stimulated the antioxidant mechanisms in mouse skin exposed to fractionated γ-irradiation resulting in a significant rise in the activity superoxide dismutase enzymes in mouse skin.

Catalase is an enzyme present in most of the aerobic cells, it protects them from oxidative stress by catalyzing the rapid decomposition of hydrogen peroxide (H2O2) in two types of reactions depending on its peroxidatic and catalatic activities (Al-Abrash et al., 2000). Our results indicated that curcumin increase catalase levels in treated cells than untreated cells this (El- Bahr, 2015) result is in accordance with the results of that showed that the levels of antioxidant enzymes SOD and catalase were significantly increased in AFB1-intoxicated rats treated with curcumin.
compared to untreated AFB1- intoxicated. Also the results of (Desai et al., 2011) showed significant increase in activities of SOD, catalase and GPx in presence of curcumin on studying the antioxidant effects of curcumin on rat peripheral blood lymphocytes (RPL) in culture. These results showed that curcumin possesses antioxidant which may be explained by the presence of two benzene rings, with one ring having a phenol hydroxy and the other ring having a phenol methoxy.

In conclusion, the present study showed the effect of curcumin as a natural antioxidant compound that enhanced the antioxidant defense system in HepG2 cells by increasing the activity levels of antioxidant enzymes SOD and catalase and decreasing the concentration of L-MDA suggesting that curcumin may be developed as anti-cancer drug in treatment of human hepatocellular carcinoma.

References:


