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Investigation of various oxidative responses of mice lung tissue after curcumin exposure

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Abstract: Curcumin is the most active compound of curcuma and it is insoluble in water and it composes about 2 to 8 percent of the weight of curcuma and the most important effects of it are anti-inflammatory and antitumor and antioxidant application. The purpose of this study is to survey the effects of Curcumin on the rate of the activities of the antioxidant enzymes and production of damage biomarkers in the lung tissue in the in vitro system. The lung tissue of a rat Balb/C has been used for this matter. In seven experimental groups and with three times of repetition under the effect of various concentrations Curcumin Monomer was incubated for 48 hours. The concentrations that were used were 0, 10, 25, 50, 100, 150 and 200micro molar. After homogenizing the changes of the activities of the catalase antioxidant enzymes, superoxide dismutase, and the amount of damage biomarkers (malondialdehyde, Dityrosine and 8-hydroxyguanine) were measured by biochemical methods. The activity of catalase and superoxide dismutase enzymes increase up to the concentration of 25 µM, compared to the control and in upper concentrations decreased. The amount of Malondialdehyde and Dityrosine up to the concentration of 50 µM decreased compared to the control. The amount of 8-hydroxyguanine, up to the concentration of 50 µM, did not changed compared to the control And they increased after this concentration. According to the obtained results, it was specified that the medicine of Curcumin with reduce of the free radicals of the cell cause reduce of damage biomarkers and increase of the activity of the antioxidant enzymes, but in the high concentration they play a toxic role in the cell.

Key words: *Antioxidant enzymes; Curcumin; Damage biomarkers*

1. Introduction

Crystalline powder of Curcumin, with a yellow – orange color, is the most active compound of curcuma. And it is practically insoluble in water but it is soluble in polar solvents. The chemical structure of Curcumin is ($C_{21}H_{20}O_6$) which was discovered by Lamp and Milobedeska, 1910 (Bharat et al., 2006).

Phenol Curcumin with the chemical formula [1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione] constitutes about 2 to 8 percent of the curcuma's weight (Aggarwal et al., 2007). Curcumin is stable in environments with physiological pHs and anti-inflammatory, antitumor and antioxidant are the most important effects of it (Bharat et al., 2006). In this study the effects of Curcumin on the activities of the antioxidant enzymes and the production of oxidative damage biomarkers and the probable mechanism of the Curcumin is been studied by an antioxidant view.

Oxygen free radicals: they are very active molecules that are able to create so many damages to the tissue, in addition to attacking unsaturated fatty acids, DNA nucleotides, and protein disulfide bonds (Adams and Odunze, 1991; Bakonyi and Radak, 2004).

The production places of the free radicals include all of the components of the cell, including mitochondrial, lysosomal, core, endoplasmic reticulum and plasma membrane. The free radicals have unpaired electron in the outer orbital layer, therefore they are unstable and highly active. These radicals take electron from other cellular constituents and make them unstable too and turn them into free radical. The antioxidants are the givers of the electron to the free radicals and they prevent the damages caused by free radicals by making them stable (Bakonyi and Radak, 2004). The produced free radicals in the cell or active species of oxygen (Reaction oxygen species) are: Hydroxyl radical (OH °), superoxide anion (O_2°) and hydrogen peroxide (H_2O_2) (Dezwart et al., 1999).

Antioxidants: they are reducing agents that can also act as pro-oxidants. The antioxidant compounds have two kinds, enzymatic and non-enzymatic. Their enzymatic kinds are (Vishal et al., 2005):

Superoxide dismutase (SOD): they are a group of associated enzymes that catalyzes the breakage of superoxide anion into oxygen and hydrogen peroxide. SOD exists in all of the aerobic cells and in the extracellular fluids. Superoxide dismutase enzymes have metal ion factors that, depending on its isozyme, can be copper, zinc, manganese or iron.

Catalase: it is a cytoplasmic enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2) by

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using iron and manganese factors. This protein is found in most of the Eukaryotic Cells. Catalase protects red blood cells against the attacks caused by hydrogen peroxide. Since hydrogen peroxide is rapidly released, in this sense, the erythrocytes can protect other tissues by absorbing hydrogen peroxide.

Glutathione peroxidase: it is a tetramer protein which has four selenium atoms. Selenium atom is necessary for the catalytic activity of the enzyme. These enzymes exist in cytosol as well as mitochondria of the animal cells, in addition to reacting with the oxidized H_2O_2, and then it is revived to the primary state with the help of glutathione. The revival power of this associated enzyme depends on the amount of NADPH (Atawodi, 2005; Bansal and Bilaspuri, 2011; Wang et al., 2007).

Lung: it is the only respiratory organ which includes two parts, left lung and right lung. From the perspective of histology the alveolar wall (pneumocyte) is composed of three main types of cell: squamous cell, large alveolar cells, and macrophages. This tissue is among tissues that produce antioxidant enzymes. The effect of the usage of Curcumin on this antioxidant enzyme has been studied (Anthony and Mescher, 2009).

2. Material and methods

2.1. Animals (a source of cells and tissue)

Healthy, mature, small laboratory mice Balb/C, 4 to 6 weeks with the weights, approximately 25 to 30 grams, male or female, were purchased from the Pasteur Institute of Iran.

2.2. Preparation of lung tissue vitro (MLCM)

Considering the methods that have been used by Burgess and et al, it was done as follows. The rats were killed by ether. The skin of the abdomen and chest were sterilized by 75% alcohol and Betadine and they were cut with the help of sterile surgical instruments under the sterile conditions. The lungs were separated from the end part of bronchi and they were removed from the chest and transferred into a petri that contained a sterilized physiology serum and they were washed in the physiology serum at least three times. The lung lobes were cultivated in the petri that contained a vitro and they were cut to pieces and were ready for distribution in the cultivation petri. About 5 grams of the lung pieces (about a pair of lung) was added to each petri with 3ml vitro DMEM 1:1 diluted of stock. These pieces were chopped into very small dimensions by surgical scissors and sterile forceps under sterile conditions. The cultivations were incubated for 48 hours in the temperature of 37 °C, CO_2 and complete humidity.

Measuring protein: in order to measure protein, a modified method *Lowry* is used (Lowry et al., 1951).

2.3. Determination of superoxide dismutase activity

It was determined by using the method of Wang et al., 1991. Finally the total activity of the superoxide dismutase enzyme was measured biochemically. Dicoumarol was put in the mixture in order to prevent the revival by pyridine nucleotides and in order to achieve the revival of NBT which is associated with oxygen. One unit of the superoxide dismutase is specified in the form of the amount of the enzyme that has done 50 percent of the prevention of NBT's revival under the conditions of experiment (Winterbourn., et al 1975).

2.4. Determination of the activity of the catalase enzyme

Activity of the catalase enzyme was determined according to the method of Ghazi et al., 2004 in the solution of the reaction of hydrogen peroxide 0.1 mM potassium phosphate buffer (pH = 7) 0.2 mM. The reaction was begun by adding enzyme extract and the value of hydrogen peroxide's breakdown was determined by reducing the absorption at a wavelength of 240 nm. Finally, the value of the activity of superoxide dismutase, catalase and glutathione peroxidase enzyme was expressed in the form of unit per milligram of protein (Habig and Jakoby, 1981).

2.5. Determination of the activity of the glutathione peroxidase enzyme

The value of the activity of the glutathione peroxidase enzyme was determined in accordance with the method of Tapple et al., 1987. The reaction was begun by adding hydrogen peroxide and the speed of oxidation of NADPH was determined by using the determination of absorption at a wavelength of 340 nm (Aebi, 1984).

2.6. Determination of the concentration of MDA and DT

In order to determine the lipid peroxidation product (MDA), the method of Satoh (Satoh, 1978) was used. Determination of the concentration of Dityrosine was measured by the HPLC method.

2.7. Methods and tools for data analysis

Each experiment was repeated three times. The average and standard deviation of the results were determined. The comparison of the results in terms of significance was done by the SPSS test by the ANOVA method and in the significance level of $p < 0.05$.

3. Results

The value of the activity of the superoxide dismutase enzyme that has been extracted from the lung tissue and under the influence of various concentrations of Curcumin monomer is increasing and it reaches the most value among the concentrations that were being tested at the concentration of 25 µM, which is a significant increase in comparison with the control. In the higher concentrations that that, the value of the activity of the enzyme decreases and it reaches the lowest value at the concentrations 150 µM and 200 µM, which is a significant decrease in comparison with the control.

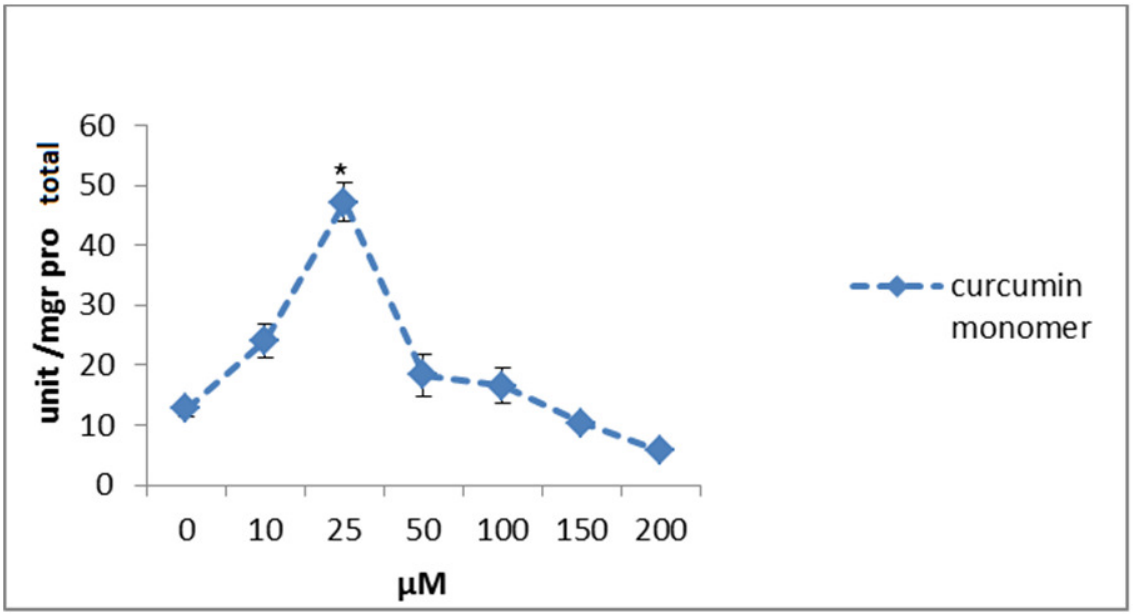

Fig. 1: various values of the superoxide dismutase caused by the effect of various concentrations of Curcumin monomer in the lung tissue. The quantities are in average ± standard deviation. The difference of the quantities has been studied in the significance level of $P < 0.05$. * Significant difference in comparison to the control

The value of the glutathione peroxidase enzyme, which is the result of the extraction of the lung tissue in various concentrations of Curcumin monomer, decreases. At the concentrations of 150 and 200 µM, the rate of decrease is 6 times compared to the control, which has the maximum decrease in comparison with other activities that have been measured in various concentrations, which statistically has a significant difference compared to the control in the significance level of $P < 0.05$.

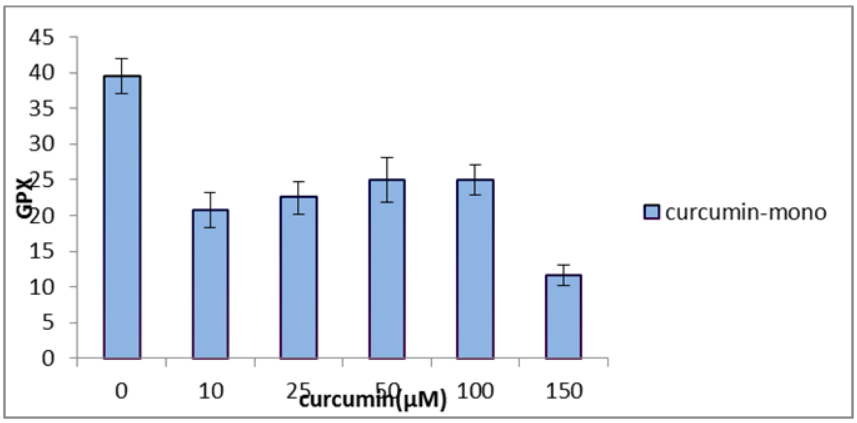

Fig. 2: The value of the glutathione peroxidase in the lung tissue under the influence of Curcumin monomer in various concentrations. The quantities are in average ± standard deviation. The difference of the quantities has been studied in the significance level of $P < 0.05$

Studying the effects of the Curcumin (monomer) medicine on the rate of the activity of the catalase enzyme was done in the vitro of the lung tissue of a rat in the various concentrations of the medicine, and at the end of cultivation, the value of this enzyme was measured. The activity of the catalase enzyme at the concentrations of 10 and 25 µM of the Curcumin monomer medicine increases and it reaches the maximum rate of activity in comparison with other observed activities, which is (54.48 ± 4.9) at the concentration of 25 µM. After that the amount of cultivation is reduced and finally, at the concentration of 200 µM of the Curcumin monomer medicine, the rate of the catalase enzyme reaches the

minimum of its value, among the observed activities, which is (10.22 ± 0.52). It statistically has a significant difference in comparison with the control in the significance level of P < 0.05.

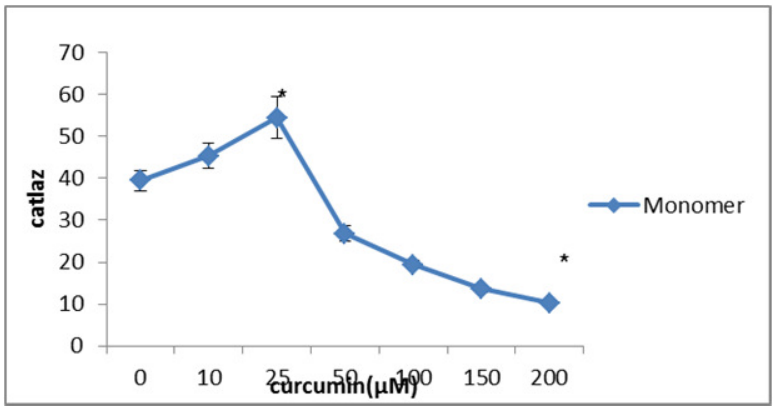

Fig. 3: The value of the catalase enzyme in the lung tissue under the influence of the Curcumin polymer or monomer in the various concentrations. The quantities are in average ± standard deviation. The difference of the quantities has been studied in the significance level of P < 0.05

The value of Dityrosine under the influence of various concentrations of the Curcumin monomer reduces at first and up to the concentration of 25 µM, the value of it reduces uniformly. After this concentration the value of Dityrosine increases more and more. It reaches the maximum of the observed value in the vitro environment at the concentration of 200 µM, which statistically has a significant difference in comparison with the control in the significance level of P < 0.05.

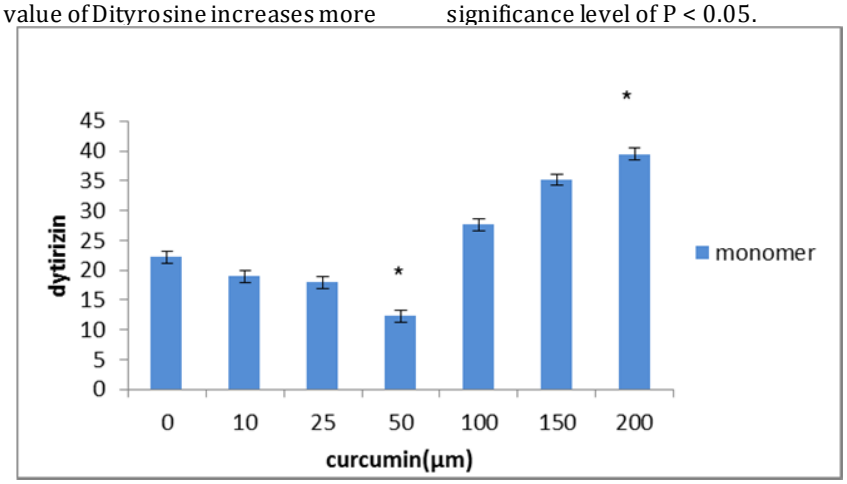

Fig. 4: The value of Dityrosine after the end of the incubation in various concentrations of the Curcumin monomer medicine in the lung tissue. *Significant difference in comparison to the control. The quantities are in average ± standard deviation

The value of malondialdehyde under the effect of the Curcumin monomer medicine after the extraction from the lung tissue decreases, in comparison with the control. The curve of the value of malondialdehyde reduces with a steep up the concentration of 50 µM, which reaches the minimum value of the malondialdehyde among other values in this concentration which is (22.2 ± 1.3). This value has reduced two times compared to the control ad has a significant difference in comparison with the control in the significance level of P < 0.05. by increasing the concentration of the medicine, the value of the malondialdehyde increases as well and finally, at the concentration of 200 µM,, the rate of it reaches the minimum of its value, among the observed activities, which is (75.6 ± 3.81), which is 1.5 times more than the control. It statistically has a significant difference in comparison with the control in the significance level of P < 0.05.

As it has been shown in the Fig. 6, the values of 8-hydroxy-nucleotide (guanine) at a concentration of 10 µM and 25 µM does not show noticeable and considerable changes in comparison with the control and it is nearly equal to the value of the control. At the concentration of 50 µM under the influence of the Curcumin monomer medicine the value of 8-hydroxy, which is (66/0 ± 5/15) increases. This biomarker just reduces at the concentration of 50 µM that this reduce is statistically significant in the significance level of P < 0.05.

4. Discussion

Antioxidants cause the destruction of the free radicals and the repair of damaged cells. The lung

tissue is among those kinds of tissues that are in contact with blood and external and environmental factors, therefore, it is rich with antioxidant enzymes (Oruc and Usta, 2007; Anthony and Mescher, 2009). During the time of their being, these enzymes are under the influence of various chemical and biological substances. Curcumin is among substances that have had medical usage and it has not been studied yet in terms of antioxidant enzymes and damage biomarkers on the lung tissue. In this research it has been studied in terms of these matters.

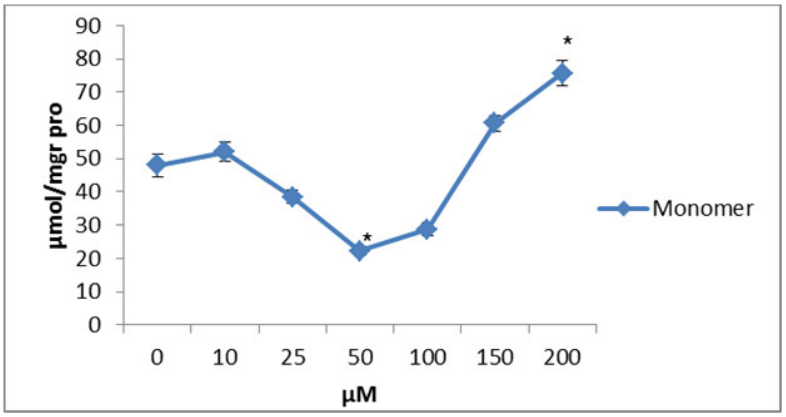

Fig. 5: The value of the malondialdehyde extracted from the lung tissue under the influence of the Curcumin polymer or monomer in the various concentrations. The quantities are in average ± standard deviation. The difference of the quantities has been studied in the significance level of $P < 0.05$. *significant difference compared to the control The quantities are in average ± standard deviation

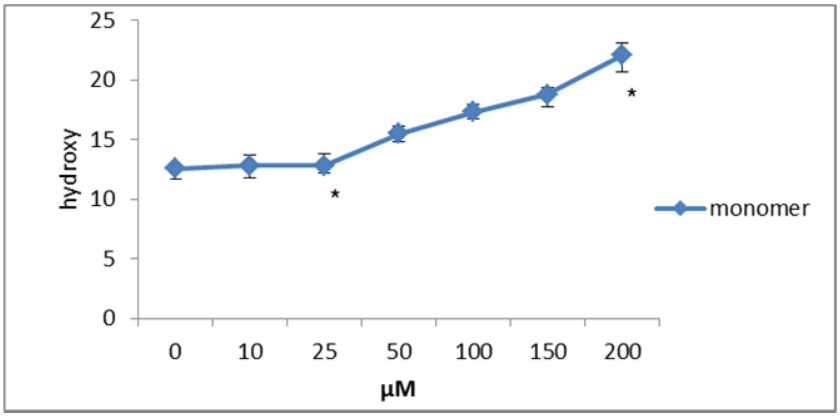

Fig. 6: The value of 8-hydroxy caused by the lung tissue under the influence of various concentrations of the Curcumin monomer medicine. The quantities are in average ± standard deviation. The difference of the quantities has been studied in the significance level of $P < 0.05$. *significant difference compared to the control The quantities are in average ± standard deviation

According to the data that has been obtained from this research, the activity of the superoxide dismutase and catalase antioxidant enzymes increases under the influence of Curcumin monomer up to the concentration of 25 µM. after this, the result is reversed and the activity of these enzymes decreases. And about the glutathione peroxidase enzyme, this curcumins cause the activity of this enzyme to decrease at all of the concentrations. The results of this research also showed that the value of the damage biomarkers Dityrosine and Malondialdehyde and 8-hydroxy-nucleotide (guanine) which are produced, respectively, from the destruction of proteins, lipids and nucleic acid due to the effect of oxidations, decreases up to the concentration of 50 µM, under the influence of Curcumin monomer and it reaches its minimum value in comparison with the control and the concentration range that is being experimented. And statistically it is significant in the significance level of $P < 0.05$. At the concentrations higher than this one, the value of these biomarkers increases. This means that this medicine prevent the destruction of macromolecules up to a certain concentration and higher than that concentration is indicative of the toxicity of this medicine on the macromolecules.

The obtained results of this research are in compliance with the previous studies in which the effects of the antioxidants activities have been studied. We can mention the study of B. Aggarwal et al on hemoglobin as one of these studies. They reported that antioxidant property of Curcumin is 10 times more, compared to vitamin E, when the glutathione of the cell reduces. This antioxidant property of Curcumin depends on the phenolic group and methoxy group on the phenyl ring of its

chemical structure. It seems that 1 and 3 D-ketone in the structure of Curcumin is important in the effects and properties of Curcumin. And also, it shows that when the phenolic group and methoxy group are in the ortho position, the activity of Curcumin increase (Bakonyi and Radak , 2004). Saibal K. et al .,2005 reported that plays the role of the antioxidant with the increase of the GSH level and glutamyl cysteine ligase (active subunit expression of mRNA) and also by reacting with the anion (O_2^-) and hydroxyl radical, thus it is effective on improving the inflammatory diseases of the lung. The antioxidant effects of Curcumin in deactivating the free radicals have been proven, but these effects often depend on the dose and environmental conditions (Dezwart., et al 1999; Weber., et al 2006).

Therefore, the results of this study show that the probable reason for the increase of the activity of the antioxidant enzymes in the lung tissue of a rat in the presence of a certain concentration of this medicine can be the reason that ROS reduces in the lung tissue, which causes the maintenance of the cell membrane fatty acids and it decreases malondialdehyde and also maintains the proteins of the cell and antioxidant enzymes and reduces Dityrosine as well as the stability of the nucleic acids of the cell.

And about the toxicity of curcumins at higher concentrations than the index concentrations that have been mentioned, some studies have been done which confirm our results. In the research of A. Baun at el., 2004, on xenobiotics, they expressed that some of the substances have a specific chemical structures such as phenols, aliphatic chlorine and naphthalene group, which play the role of a toxic property in the cell (Baun., et al 2004). By taking this research and the chemical structure of Curcumin, which has a phenolic group, into consideration, it can be said that this medicine has toxic properties in the cell at the concentrations that are higher than the index concentrations, in such way that it causes the decrease of the activity of the antioxidant enzymes and increase of the damage biomarkers.

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