STUDY OF ANTIOXIDANT ACTIVITIES OF EUFORBIN POLYPHENOL

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ABSTRACT

Polyphenols are involved in metabolic processes in the body, exhibit antioxidant and antioxidant properties at the expense of ON groups, and low levels of toxicity can lead to a number of problems in medicine, food, cosmetics, pharmaceuticals and manufacturing [Duthie et al., 2000].

Therefore, it is necessary to study the mechanism of action of polyphenols at the tissue and cellular levels, and to determine their pharmacological properties, and the advances will help to keep the genome of the population healthy and healthy. The results will lead to promising advances in pharmacology, medicine, physiology, and biophysics [Парфенов., 2007].

Purpose of the study:

Study and pharmacological evaluation of antioxidant, anti-radically and anti-hypoxanthic properties of Euforbin polyphenol in vitro and in vivo experiments.

Materials and methods of research:

In carrying out this work, presented by the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan: Euphorbia ferganensis B. Fedtch (Drying) (1-O-galloil-2,4-valoneil-3,6-hygsahydroxydifenol-β-D-glucose pharmacological properties of glucose) polyphenol have been investigated.

The antioxidant activity of Euforbin polyphenol was evaluated in the context of the effect of hepatic mitochondria on lipid peroxide oxidation process in non-white white rats with a body mass of 120–140 g. Mx was isolated from the liver of rats by differential centrifugation [Schneider, et al., 1951]. Dissociation medium: 250 mM sucrose, 10 mM tris-chloride, 1 mM EDTA, pH 7.4.

Determination of lipid peroxide oxidation products in mitochondria

LPO products were studied in the presence of thiobarbiturate acid (TBA). The reaction was stopped by adding 0.220 ml of 70% trichloric acid to IM. After this phase, the mitochondria suspension was centrifuged at 4,000-minute intervals for 15 minutes. Then, 2 ml of precipitate was removed and 1 ml of 75% TBA was poured. 2 ml of N_2O and 1 ml of TBA were added to the control test tube. The mixture was incubated for 30 minutes in a water bath. After cooling, a change in optical density at a wavelength of 540 nm was detected.

In calculating MDA, extraction with the molar coefficient (e=1,56ч10^5 M^{-1} $sм^{-1}$) was used: nmol MDA / mg protein = D / 1,56x30.

The amount of protein in the mitochondria was calorimetrically determined by the Biuret method. Cattle whey album was used as the standard.

Fe^{2+}/ ascorbate system was also used to study the LPO process in the mitochondria membrane. Under the influence of this system, the mitochondrial membrane loses its barrier function, resulting in an increase in organelle volume and a loss of mitochondria. This

volume change was determined by photometric change in optical density at 540 nm wavelength in 3 mL cells. IM: KSL - 125 mM, tris-NSL - 10mM, (pN 7.4); Concentrations: $FeSO_4$ - 10 μM, ascorbate - 600 μM; the amount of mitochondria is 0.5 mg / ml [Almeida., 2006].

The obtained results and their analysis

Antioxidant drugs improve the physiological and functional system of the cell, increasing the stability of damaged cells and mitochondria membranes. The antioxidant property of Euforbin polyphenol was assessed by investigating the process of peroxide oxidation of lipids resulting from the induction of rat liver mitochondria under the effect of Fe^{2+} / ascorbate [Almeida et al., 2006].

In the experiments, the addition of 10 μM $FeSO_4$ and 600 μM ascorbate to the incubation medium had an inducible effect on the mitochondrial membrane structure, resulting in permeable membrane permeability. Under these conditions, membrane permeability increased by 45.3 ± 1.76% as a result of structural disturbance of the Mx membrane. In the experiments, the Mx membrane permeability values were calculated and were 100%. Increased membrane permeability under Fe^{2+} / ascorbate evidence indicates that the LPO process is formed in Mx. In subsequent experiments, the correction effect of Euforbin polyphenol on LPO was analyzed. Adding 1 μM of Euforbin together with Fe^{2+} / ascorbate to the incubation medium reduced the peroxide oxidation process of lipids in Mx by 2.0% ± 1.2. This is the first indication that Euforbin polyphenol has antioxidant properties. The antioxidant effects of Euforbin have been studied in greater detail by increasing the concentrations added to the incubation medium. 3 μM of Euforbin added to the medium inhibited the LPO process at Mx by 20.6% ± 1.2, 5 μM by 63.6% ± 1.5, with 6 μM by 87.9% ± 0.5, and 92.3% ± 0.5 by 8 μM. Euforbin at 10 μM showed a high antioxidant property by inhibiting the LPO process in Mx to 100%. In the experiments, the half-maximum inhibitory concentration of Euforbin (IS50) was 3.72 μM.

The study of the activity of the studied substance to determine the antioxidant effect is fully covered by the study of the amount of MDA produced by the LPO process. Because, under pathophysiological conditions, the main disorders in tissues and cellular antioxidant systems

are the degree of qualitative and quantitative changes of lipids by the process of production of LPO products (MDA). In addition to enhancing the membrane stability of the anti-oxidant pharmacological agent Mx, it is also essential to prevent MDA secretion from the LPO process. Only then, the antioxidant activity of the investigated substance is fully covered.

In our experiments, MDA release in the LPO induced by Fe^{2+} / ascorbate effect (control group) was 3.52 ± 0.18 3.2 nmol / mg of protein.

Adding 1 μM and 5 μM concentration of Euforbin in the incubation medium did not significantly affect the accumulation of MDA in the membrane. The major quantitative change in the MDA decay occurred at a concentration of 20 μM of Euforbin. Under the effect of 5 μM of Euforbin, the MDA in the membrane was 2.98 nmol / mg of protein, 2.1 nmol / mg of protein at 15 μM, and 1.8 and 0.7 nmol / mg of protein at 15 and 20 μM, respectively. In this experiment, the highest inhibitory concentration of Euforbin was 25μM and the MDA content was 0.46 nmol / mg of protein.

The results of the experiments prove that Euforbin is a pharmacological agent with high antioxidant properties. MDA inhibited the decomposition process by preventing the increased LPO process induced by Euforbin Fe^{2+} / ascorbate action.

REFERENCES :

1. Парфенов Э.А. Физиологически совместимые антиоксиданты как средства самозащиты животных организмов // Мат. пятой нац. конф. «Активные формы кислорода, оксид азота, антиоксиданты и здоровье человека». –Смоленск: ФГУ «Смоленский ЦНТИ», 2007. – С.125-127.
2. Almeida A.M., Bertoncini C.R. Mitochondrial DNA damage associated with lipid peroxidation of the mitochondrial membrane induced by Fe2+-citrate // An. Acad. Bras. Cienc. –2006. – 78. – P.505-514.
3. Duthie G., Crozier A. Plant-derived phenolic antioxidants // Curr. opin. lipidol. – 2000. – V. 11. – P. 43-47.
4. Schneider W.C., Hogeboom G.H. Cytochemical studies of mammalion tissues: the isolation of cell components by differential centrifugation // Cancer. Res. – 1951. – 11. – P. 1-22.