

The Protective Effect of Aqueous Extract of Broccoli on the Levels of Glutathione in the Serum of Pregnant Mothers During (7,16,20) Days of Pregnancy Treated with Carbamazepine

Nawras Ali Hussein Al-Jubouri¹, Dalal Abdul Hussein Kazem Al-Issawi²

^{1,2} University of Kufa /Faculty of Education for girls Department of Biology/Iraq

Abstract

The study was performed at the animal house of Biology department, College of Education for girls and included the use of 75 laboratory rat animals of Sprague-Dawley strain, randomly divided into five equal groups: the first group was administrated with distilled water (negative control), the second group was orally administrated with Carbamazepine drug 200mg/kg/day, the third group was orally administrated with aqueous extract of Broccoli (*Brassica oleracea* L. var. *Italica*) 1500 mg /kg/ day + Carbamazepine drug, the fourth group was orally administrated with aqueous extract of Broccoli (*Brassica oleracea* L. var. *Italica*) 2500 mg /kg/ day + Carbamazepine drug, the fifth group was orally administrated with aqueous extract of Broccoli (*Brassica oleracea* L. var. *Italica*) 3500 mg /kg/ day + Carbamazepine drug. The treatment of all groups (experimental animals) was once daily (1- 7, 1-16, 1-20 day of pregnancy). The results of the statistical analysis of the current study showed that there was a significant decrease ($P < 0.05$) in the levels of glutathione (GSH) in the serum of pregnant mothers treated with carbamazepine 200 mg / kg when compared with the control during the three stages of pregnancy (7,16,20), while the results showed Statistical analysis: There were no significant differences ($P > 0.05$) in the levels of glutathione (GSH) in the serum of pregnant mothers in the treated groups (aqueous extract of broccoli plant 1500 mg/kg, carbamazepine 200 mg/kg, aqueous extract of broccoli plant 2500 mg/kg, carbamazepine 200 mg/kg, aqueous extract of broccoli plant 3500 mg/kg, carbamazepine 200 mg/kg) in comparison with the control group during the three stages of pregnancy (7,16,20). We conclude from the current study that broccoli has a role in preventing the side effects of carbamazepine drug, so the pregnant mother should eat broccoli during pregnancy.

Keywords: Carbamazepine; pregnant mothers; glutathione

1. Introduction

Medicinal plants are important for human life, due to their importance in getting rid of toxins, and most of them are associated with no or low side effects. Many medicinal plants showed a protective effect on the liver and kidneys in disorders caused by paracetamol, gentamicin, profenofos, toxic drugs, chronic pressure, diabetes and nephrolithiasis chemically caused by oxidative stress and inflammatory mediators (Nazir et al, 2021).

(Hamza, 2020) mentioned that the emergence of herbal medicine dates back to about 6000 years, the ancient Egyptians and Pharaohs were among the first peoples to take care of medicinal plants, and the Chinese collected medicinal plants and used them since 4000 or 5000 years BC, but the discovery and widespread use of antibiotics in the last century, it led to a decline in the use of medicinal and aromatic plants and herbs, and given the limited use of these antibiotics due to their side effects and the resistance of some microorganism strains, medicinal and aromatic plants and herbs regained their position as sources of important medicines, and were also used as flavorings and preservatives in some countries of the world because of its presence in nature and its possession of many active groups with high efficacy

and wide use, as well as the limited side effects it causes.

The broccoli (*Brassica oleracea* var. *Italica*) is an annual herbaceous plant similar to cauliflower in its external appearance. It is one of the types of medicinal plants found in the Mediterranean region. It is also characterized by its high nutritional value. It is a non-fat, sodium and calorie-free food, and it is a good source of many vitamins, Such as vitamin D, A, C, folic acid, niacin, riboflavin and carotenoids, and it also has high therapeutic properties as it is a strong regulator and antibiotic for many widespread diseases, as it works to reduce the level of cholesterol in the blood and regulate the level of sugar in the blood and reduce high blood pressure and helps in increasing physical strength and building bones, in addition to that, it helps in preventing heart diseases, genital and urinary diseases, and reduces the incidence of cancer, as well as a rich source of anti-cancer glucosinolates (GIs), as it confirmed that eating more than one meal during the week reduces the risk of cancer by 45%, in addition to that it works to prevent retinal diseases (Al-hussainy and Manea, 2019).

Carbamazepine or Tegretol is a drug used to treat epilepsy as well as analgesic for nerve pain and reduces symptoms of mania, in addition to being used as an adjuvant drug in the treatment of

schizophrenia with other drugs intended for this purpose (Whalen, 2015).

2. Materials and Methods

2-1 Plant material

Plant collection

The broccoli plant used in the current study was brought from the local markets in Al-Najaf Governorate, and it was diagnosed and classified by Dr. Rokaya Menon in the College of Education for Girls / biology department.

Extract preparation

After cleaning the broccoli plant and washing it with filtered water well to remove all suspended impurities, it was cut and left to air dry at room temperature in the shade until it becomes dry. After the drying process, it was ground with a household electric mill to obtain powder (Somasundaram et al, 2018). The hot aqueous extract was prepared using the Soxhlet apparatus And by 1000 ml distilled water (instead of alcohol), then weighing 60 gm of the dry matter powder of the broccoli that was wrapped in filter paper and placed in the extraction thimble of the Soxhlet apparatus for 24 hours, and the process was repeated several times in order to obtain an appropriate amount of the active substance (Ahmed and Rao, 2013). The samples were dried by placing them in sterile containers and exposing them to air only, after which they were collected, weighed, and kept in the refrigerator until use.

2-2 Carbamazepine drug

In this study, the drug Carbamazepine was used, which was obtained from pharmacies in Al-Najaf Province and produced in Cyprus by Medochemie LTD-cyprus (Europe), which is used as an antiepileptic treatment. The active substance is 5 H-dibenz [b,f] azepine- 5- carboxamide, each tablet contains 200 mg. The tablets are white, round and flat, as is the case with other antiepileptic drugs, and the concentrations were prepared according to the weights of the animals.

2-3 Animal

The 75 Albino rats were brought from the University of Karbala/College of Pharmacy at the ages of 3 months and weights (190-250)g . It was exposed to a light cycle, 12:12 hours light: dark and temperature 25c° , give them adequate food and water continuously during the experiment . The animals were left for two weeks to adapt to the place and to make sure they were free of diseases and not pregnant . Then two sexually mature females were placed with one sexually mature male in one cage throughout the night , the mating was confirmed by observing the male sperm in the vaginal swab and also by observing the vaginal plug , the day observed for sperm in the vaginal swab or vaginal plug is zero day of pregnancy and the next day is the first day of pregnancy . The animals were divided into five

groups:-

Group 1/ It included (15) pregnant female rats who were dosed orally with distilled water from the first day of pregnancy until the end of the experiment. This group was divided into (5) females who were sacrificed on day (7) of pregnancy and (5) females who were sacrificed on day (16) from pregnancy and (5) females sacrificed on the (20) day of pregnancy. This group was considered a negative control group.
Group 2/ included (15) pregnant female rats who were orally dosed with carbamazepine 200 mg/kg from the first day of pregnancy until the end of the experiment, and this group was divided into (5) females who were sacrificed on day (7) of pregnancy and (5) females who were sacrificed on the (16) day of pregnancy and (5) females sacrificed on the (20) day of pregnancy

Group 3/ It included (15) pregnant female rats that were dosed orally with an aqueous extract of broccoli at a dose of 1500 mg/kg first, and an hour after giving the dose, they were dosed orally with carbamazepine 200 mg/kg once daily from the first day of pregnancy until the end of the experiment. This group was divided into (5) females sacrificed on day (7) of pregnancy, (5) females sacrificed on day (16) of pregnancy, and (5) females sacrificed on day (20) of pregnancy

Group 4/ It included (15) pregnant female rats that were dosed orally with an aqueous extract of broccoli plant at a dose of 2500 mg/kg first, and an hour after giving the dose, they were dosed orally with carbamazepine 200 mg/kg once daily from the first day of pregnancy until the end of the experiment. This group was divided into (5) females sacrificed on day (7) of pregnancy, (5) females sacrificed on day (16) of pregnancy, and (5) females sacrificed on day (20) of pregnancy

Group 5/ It included (15) pregnant female rats that were dosed orally with an aqueous extract of broccoli plant at a dose of 3500 mg/kg first, and an hour after giving the dose, they were dosed orally with carbamazepine 200 mg/kg once daily from the first day of pregnancy until the end of the experiment. This group was divided into (5) females sacrificed on day (7) of pregnancy, (5) females sacrificed on day (16) of pregnancy, and (5) females sacrificed on day (20) of pregnancy.

2-4 Anatomy of rat females and blood collection

After a day had passed since the last dose of the experiment and the end of the dose period for all groups, the weights of the female rats after the treatment, which had previously been recorded before the treatment, were recorded during each of the three pregnancy periods (7, 20,16) days, respectively, and for the different treatments, using the weighing scale, then the animals were anesthetized using a mixture of xylazine (0.6) and ketamine (0.4), which equals (1 ml), meaning (0.2) for each animal, then a blood sample was withdrawn from the heart directly through a heart puncture to

obtain the largest amount of blood, of course, after fixing the animal on the dissection plate with pins and it was (5 ml) blood, then the blood samples were placed in Gel tubes free of anticoagulant, then the tubes were left for (15-20) minutes at the laboratory temperature. After that, the tubes were placed in a centrifuge for the purpose of separating blood serum, then the serum was transferred to sterile and clean test tubes and kept at a temperature (-20 ° C) until the biochemical analyzes included in this study are conducted (Boskabadi et al,2013).

2-5 Estimation of glutathione (GSH) levels in blood serum

The principle

Reduced glutathione (GSH) levels were measured by using the optimal enzymatic cycling method by using reduced glutathione to quantify glutathione (Tietz, 1999). This method focused on the action of the sulfhydryl group, where the chromogenic (5,5-di) Thio-2-nitro benzoic acid) through the sulfhydryl group of the glutathione molecule to the yellow compound 5 - thio - 2 - nitro benzoic acid TNB 5 - in an intense form, and then the absorbance of the reductant was measured at length. The wavelength is between (405-414) nanometers.

Reagents

1-GSH mes buffer

The buffer solution contains (0.4) molar of (N-morpholine ethanesulphonic acid)², (100) molar of phosphate and (2) micro-molar of ethylenediamine tetra acetate (EDTA).

2- GSSG standard

3- GSHCO – factor mixture

4- GSH enzyme mixture

The procedure

1- Add 50 microliters of standard solution to each

compartment.

2- Add (50) microliters of sample to each compartment.

3- The plate is covered with the designated cover.

4- The mixture is prepared for the examination by mixing the following reagents (estimating buffer solution (11.25) ml, the mixture for the cofactor (0.45) ml, the mixture for the enzyme (2.1) ml, in addition to a solution of 5.5 - di-NiO₂ - nitrobenzoic acid 0.45 ml).

5- The cover of the plate is removed and (150) microliters of the test mixture that was prepared is added to each of the compartments that contain the standard solutions and samples, then the cover is returned to the plate and the darkness is incubated on the vibrating orbital shaker.

6- After that, the absorbance was measured at a wavelength of 405-414 nm, and this was done after about (25) minutes.

3. Results

The results of the statistical analysis of the current study shown in table (3-1) showed that there was a significant decrease ($P < 0.05$) in the levels of glutathione (GSH) in the serum of pregnant mothers treated with carbamazepine 200 mg / kg when compared with the control during the three stages of pregnancy (7,16,20), while the results of Statistical analysis showed that there were no significant differences ($P > 0.05$) in the levels of glutathione (GSH) in the serum of pregnant mothers in the treated groups (aqueous extract of broccoli plant 1500 mg/kg, carbamazepine 200 mg/kg, aqueous extract of broccoli plant 2500 mg/kg, carbamazepine 200 mg/kg, aqueous extract of broccoli plant 3500 mg/kg, carbamazepine 200 mg/kg) in comparison with the control group during the three stages of pregnancy (7,16,20).

Table (3-1) The protective effect of aqueous extract of broccoli on the levels of glutathione in the serum of pregnant mothers during (7,16,20) days of pregnancy treated with Carbamazepine			
Groups	Stages of pregnancy (day)		
	7 day	16 day	20 day
Control Group	47.875±2.54	44.093±0.32	37.275±2.66
carbamazepine 200 mg / kg	33.042±0.51	30.369±0.59	25.732±0.53
aqueous extract of broccoli 1500 mg/kg + drug carbamazepine 200 mg/kg	47.186±2.67	43.716±2.12	38.608±0.57
aqueous extract of broccoli 2500 mg/kg + drug carbamazepine 200 mg/kg	48.151±1.99	44.267±1.44	38.610±1.14
aqueous extract of broccoli 3500 mg/kg + drug carbamazepine 200 mg/kg	47.468±2.57	43.280±0.91	37.243±1.75
LSD	4.027	2.286	2.833

4. Discussion

The results of the statistical analysis of the current study showed that there was a significant decrease in the levels of glutathione (GSH) in the serum of pregnant mothers treated with carbamazepine 200 mg / kg when compared with the control during the three stages of pregnancy (7,16,20), the reason for this result may be that a drug stimulated the formation of free radicals in the body, especially

active oxygen radicals, which are strong oxidation factors when present in high concentrations, causing oxidative stress that stimulates crash oxidative stress in the various cells and tissues of the body as a result of its intense interaction with the lipids in the cell membranes, causing oxidation of them and other important molecules such as proteins, nucleic acids, etc. Inside the cells, stimulating their destruction, and thus the cells of the various tissues in the body are destroyed, causing high levels of MDA, which is

an indication of the destruction of tissues resulting from the effects of free radicals in the body, therefore, excessive concentrations of free radicals cause the depletion of antioxidants such as GSH, which is the first line of defense against these free radicals. Various antioxidants interact with free radicals generated as a result of various oxidizing agents to rid the body of their damaging damage to body tissues, which leads to a decrease in their levels in the serum (Akhzari et al., 2019). The results of the current study agreed with what was found (Imad A. Thanoon et al., 2012). The study included 38 male adult patients with primary generalized epilepsy, on continuous CBZ monotherapy, for at least six months, It showed that there was a significant increase in the level of malondehyde and significant decrease in the levels of glutathione (GSH) in the blood serum.

The results of Statistical analysis showed that there were no significant differences in the levels of glutathione (GSH) in the serum of pregnant mothers in the treated groups (aqueous extract of broccoli plant 1500 mg/kg, carbamazepine 200 mg/kg, aqueous extract of broccoli plant 2500 mg/kg, carbamazepine 200 mg/kg, aqueous extract of broccoli plant 3500 mg/kg, carbamazepine 200 mg/kg) in comparison with the control group during the three stages of pregnancy (7,16,20), the reason may be due to the nutrients, elements and vitamins present in the plant, which had a protective effect against the effect of the carbamazepine.

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