The Protective Role of Amygdaline on the Enzyme ALP and Non-enzymatic Antioxidants and GST on the white–Albino Mice Treated with the Carbon tetrachloride compound.

Iqbal Fadhel Alwan, Zeyad Tareq Habeeb and Ammar Mawla Ahmood.
1-Ministry of Science and Technology, Iraq, Baghdad, Box, 765.
2-University of Kerbala. College of Education for pure Science.

Abstract

The study aims to evaluated the protective effect of Amygdaline compound extracted from apricot seeds on the effectiveness of Alkaline phosphates (ALP), (Glutathione –S- transfers (GST) and Non-Enzymatic antioxidant (vitamin A,E,C) in serum laboratory animals. Amygdaline compound was analyzed by High performance liquid chromatography technique (HPLC). The tests were conducted on the interference males on the albino mice treated with carbon tetrachloride were studied, 32 male albino mice Swiss albino, (5-8 weeks) and (20-25 gm weight).

Conducted tests on the overlap between the three concentration (100, 200,300 mg / kgm) of aqueous extract of Amygdaline (3.2 mg / Kgm) of carbon tetrachloride with interaction included two types of treatment (pre-CCL4 and post-CCL4) through oral dosage and for a period of 14 days.

The study shows that the concentration 300mg / kgm is the best concentration of aqueous extract there was used and study suggests that use the concentrations of this focus and fact that the plant is used for human consumption broadly and because it contains the Bioflvone.

Keywords: carbon tetrachloride, Amygdaline compound, Non-enzymatic antioxidants.

Introduction

Amygdaline is a bioflavonoid derivative of apricot nuclei. The formula of this compound is C20H27NO11. It has an antioxidant role for its ability to remove oxygen free radical and protect the cellular molecules from oxidation damage (1). Apricot kernel contains antioxidants (amygdaline) that protect cells from damage and cancer and contain abundant amounts of minerals and essential vitamins, which are important compounds in reducing the risk of cancer and help to enhance the immunity of the body and protect the weakness of the immune system associated with aging, where (2) and (3) and figure (1) shows the chemical composition of the amygdaline compound.

Figure (1) shows the chemical composition of the amygdaline.
It also activates the genes and biochemical and antacid changes of immune cells and reduces free radicals, as well as their importance in the treatment of cancer and its causes. Environmental pollutants are considered to be harmful to human health.

Among the most important problems of the day are industrial and human activity. Many chemical compounds are used for various purposes, including the manufacture of pesticides, drugs and chemical industries, including carbon tetrachloride, its aroma is light and can be identified even at a few levels. Causes a clear toxicity of liver through the destruction of its cells or damaged by several mechanisms including necrosis and liver lipid (4).

The increase in the effectiveness of liver enzymes can occur as a result of increased cell processing processes or as a response to cell growth processes. Including alkaline phosphates’ (ALP), which is used to measure the live’s extractive.

On the other hand note (5) that the antioxidants lead to stimulate the increase in the activity of the enzyme (GST) glutathione –S- transfers. This enzyme is a multi-functional enzymes by removing the toxic metabolites of some carcinogens and mantras.

Non-enzymatic antioxidants, Vitamin A is a non-enzymatic, antioxidant and immune system that has the ability to protect the body against cancer. Vitamin C is found in blood plasma, white blood cells and various body tissues and acts as an antioxidant, indicating its protective function of free radicals (6). Vitamin E dissolves fat and is one of the most important sources of antioxidant non-enzymatic and protects the body from cancer, which is important for the vitality of the body and activity of blood vessels and concentrated in the liver and heart (7).

The purpose of the study was to evaluated the protective effect of amygdaline on the effectiveness of ALP, GST and Non—Enzymatic antioxidant (Vit. A,E,C ) in serum for laboratory animals.

**Materials and methods of work:**

**Materials : preparation of the extract**

The water extract of the apricot seeds is prepared by the suxsolaite using a mixing ratio (1g:7.5ml). The mixture is then heated for 2 hours, then filtered and concentrated. Then, use the cooling device to obtain the amygdaline compound powder.

The purity of the amygdaline compound was measured and compared with a standard model equipped with Spulco. Using the Shimatza A6 High performance liquid chromatography (HPLC), column type ODS C18 and absorbance at a wave length of 251nm (8).

**Mobile phase :** A composed of non-ionic water + phosphoric acid (1000-1) volume / volume.

: B Ingredient of Acetonitrile + phosphoric acid (1000-1) volume / volume.

The program of mixing mobile phase solutions during the separation process as it passes through the separation column consists of (min) – 15%, (20min)-30%,(25min) -100%, (45min.)0%=B and the mobile phase rate of 1ml / 1min. Absorption is estimated along wavelength (UV.202nm)

**Laboratory animal management.**

In this study, 32 male Swiss albino mice at the age of 5-8 weeks and an approximate weight (20-25g) were placed in plastic cages, fed with concentrated feed and water. And divided into four groups as follows:

**The first group :**

It was composed of (4 mice) and phosphate Buffer Solution (PBS) solution was given on the first day and water for 13 days. It was killed on the 14th day by separating cervical vertebrae and negative control.
The second group:
This group involved 4 mice and CCl₄ was given orally at a concentration of 3.2 mg/kg on the first day orally and then water was given (for thirteen days) and was killed on the 14th day by separating the cervical vertebrae and Positive control.

The third group:
This group consisted of 12 mice divided into three groups, CCL₄ was given orally at 3.2 mg/kg orally, and 6 hours later, the water extract of the amygdaline compound was given three concentrates (100, 200, 300 mg/kg) for 13 days and was killed on the fourteenth day by separating cervical vertebrae.

The fourth group:
This group consulted of 12 mice divided into three groups. The water extract of amygdaline was given in three concentrations (100,200,300 mg/kg) for 13days. After that CCl₄ was given a concentration of 3.2 mg/6 hours from the end of the thirteenth dose and are dissected at the beginning of the fourteenth day by separating cervical vertebrae.

Preparation of blood solution:
Animals were killed and blood was taken from the heart of the mouse and placed in a test tube containing EDTA as an anticoagulant. Mix the solution and discard by centrifugal device at 2000 cycles/min for 10 minutes. Use leach ate to measure non-enzymatic antioxidants and biological agents.

Enzymatic Assays:
Determination of the level and effectiveness of the enzyme (alkaline phosphates) (ALP): The sample used in the this test is Serum to determine the efficacy of the enzyme (Alkaline phosphates) (ALP) and followed (9), where four test tubes are used for each model representing the first sample and the second model sample and the third represents the standard sample and the last represents the fourth control sample reagent bland and attended these models according to the instructions adopted in to measure the enzyme, mix the tubes well and place in a dark place for 5 minutes, after which the optical density of the solutions was measured along a wavelength (510nm) using the optical spectrometer.

Measurement of non-enzymatic antioxidants
The concentration of vitamin A was measured using HPLC a moving phase consisting of 99% methanol. 1% distilled water, 330nm wavelength, and column type ODS C-18.9 The concentration of vitamin C was measured using HPLC using the mobile phase consisting of dissolve sodium acetate and EDTA in distilled water with PH = 6 and 254nm and using column C-18 ODS.
The concentration of vitamin E was measured using the modified method of Deleen heer. An anti-oxidant solution was prepared with the standard solution using a moving phase consisting of 99% methanol alcohol, 1% distilled water, 287nm wavelength and column type 11C-18 ODS.

Preparation of the blood solution (to determine the level and efficacy of the GST enzyme):
Animals are killed by cervical dislocation and take blood from the heart of mouse and placed in a test tube containing EDTA as an anticoagulant. Mix the solution and discard the centrifuge at 3000 rpm for 10 minutes with a salt solution (0.9%). Leave the leach ate and add 1ml of distilled water and mix well and keep at -20° C until used to measure the enzyme (GST).
Determination of the level and efficacy of the enzyme GST:
The model used is the same as the sample used to measure the efficacy of glutathione –S-transferase, as shown in method (10).
Results were subjected to variance analysis using SAS(2001). For the least significant difference between group mice, the Least Significant Difference / LSD was used at a probability level (p< 0.05) to determine the differences between the different transaction levels.

Results and Discussion:
HPLC tests:
Figure 2 shows the separation of the amygdaline compound by the HPLC at the time of detention (16.5) minutes. Figure(3) shows the standard amygdaline compound at the time of detention (16.5) minutes, showing that they coincided with the time of detention(11).

![Figure 2](image1)

Figure 2 shows the determination of the purity of the amygdaline extract of the apricot seed extract by HPLC.

![Figure 3](image2)

Figure 3 shows the purity of the standard solution of the amygdaline compound by HPLC.

Effectiveness of GST enzyme in red blood cells:
In the present study noted a decrease in the level of efficiency of GST in the treated group with (100 mg/kg) of water extract of amygdaline compared with CCL₄ (3.2mg /kg). Either in the tressed group with (200mg/kg) of water extract (for amygdaline). A reduction was observed, more than (100mg /kg) which is evidence the efficiency of (300mg/kg) extract was found to be lower in comparison with the previous two groups(12). This is an indication of the effect of increasing the concentration of the extract on the efficiency of GST note from Fig.(4).
That the efficiency of GST may increase when the CCL₄ (induced) is compared to the negative group during the dosage period. It is shown that the use of the extract before giving the carbon tetrachloride better than carbon tetrachloride compound (induced) because the body contains antioxidant substances and also helps to increase the body's immunity. we note a decrease and improvement in the significance of the enzyme GST Which is important in the diagnosis of liver
damage compared with the untreated group because the amygdaline compound contains highly effective materials against oxidation process (13).

![Graph showing efficacy of ALP enzyme](image)

Figure (4) shows the effect of the extracted dosage of the apricot seed on the efficacy of GST for 14 days before and after the CCl₄ (induced).

A = negative control (distilled water).

B = positive control CCl₄ dose of 3.2 mg / kg.

C = extract with a dose of 100 mg / kg.

D = extract with dose (200 mg / kg).

E = extract with a dose of 300 mg / kg.

**Level of efficacy of ALP enzyme**

The enzyme ALP is present in different areas of the body such as the intestines, bone marrow, liver and kidney, but with different concentrations and a few compared with other enzymes. The effectiveness of the enzyme varies with the stimulant or inhibitory substances in the middle of the reaction,(p<0.01) of the amygdaline extract of the apricot seeds on the enzyme ALP in the blood of laboratory mice before and after the 14 –day dosage of the drug to determine the effect of CCL₄ during the duration of the dosage compared to the negative control and positive control models. Figure (5) show a decrease in the level of efficacy of the ALP enzyme in the group (100mg/kg) of the amygdaline extract compared with the treated group with CCL₄,(200mg /kg) of the extract was observed with a decrease of more than 100mg/kg. This is evidence of the efficacy of the extract. The concentration group (300mg/kg) of the extract was lower in comparison with the group (100 and 200mg/kg). This is an indication of the effect of increasing the concentration of the plant extract on the activity and efficacy of the enzyme ALP(14).

A which showed that there was a more effective effect of the extract of the of the apricot seeds plant when given before the carbon tetrachloride (induced) and not after (induced) for a period of 14 days, induction. That the plant contains effective compound have the ability to not harm any proteins that inhibit the enzymes that work together to prevent the damage caused by the process of oxidation of the contents of the cell such as nucleic acids and proteins and fat, including systems that prevent the occurrence of these types of interactions or removed before causing damage to cell contents(15).
Fig. (5) illustrates the effect of the amygdaline extract on the efficacy of the ALP enzyme for 14 days before and after the CCL₄.

A= negative control CCL₄ dose of 3.2mg/kg.
B= positive control CCL₄ dose of 3.2mg/kg.
C= extract with a dose of 100mg/kg.
D= extract with dose (200mg/kg).
E= extract with dose of 300 mg/kg.

**Non–enzymatic Antioxidants**

**Vitamin A**

The results of this study for the effect of three different concentrations of Amygdaline compound on vitamin A in the blood of laboratory rats before and after the 14-day dosage of the CCL₄ during the duration of the dosage compared with control models negative and positive control, and there were significant differences (p <0.01). In Figure (6) noted a decrease in the level of efficacy of vitamin A in the treated group (100 mg / kg) of extract (Amygdaline) compared with the treated group CCl₄ (induced). (200 mg / kg) of the extract was observed with a reduction of more than 100 mg / kg. (300 mg / kg) of the extract was lower in comparison with the group (100 and 200 mg / kg). This is an indication of the effect of increasing the concentration of the water extract of the Amygdaline on vitamin A. Which showed that there was a more effective effect of the extract of water of the Amygdaline on an enzyme when given before carbon tetrachloride (induced) and not after (induced) for a period of 14 days, indicating that the cells were containing an antioxidant and vitamin A . Before the induction is given that Amygdaline an anti-oxidative compound, although vitamin A is important in immunizing against cancer (16).

Figure (6) shows the effect of the extracted dosage of Amygdaline on the efficacy of vitamin A for 14 days before and after the CCL₄ (induced). .

A = negative control (distilled water).
B = positive control CCl₄ dose of 3.2 mg / kg.
C = extract with a dose of 100 mg / kg.
D = extract with dose (200 mg / kg).
E = extract with a dose of 300 mg / kg.

**Vitamin C**

The results of this study for the effect of three different concentrations of Amygdaline on vitamin C in the blood of laboratory mice before and after the 14-day dose of the CCL₄ (induced) compared with negative control and positive control models, there were significant differences (p <0.01). In Figure (7), noted a decrease in the level of efficacy of vitamin C in the treated group (100 mg / kg) of Amygdaline extract compared to the CCl₄ (induced) group. (200 mg / kg) of the extract was observed with a reduction of more than 100 mg / kg. (300 mg / kg) of the extract was found to be significantly lower in comparison to the group (100 and 200 mg / kg) this is an indication of the effect of increasing the concentration of water extract of Amygdaline on the activity and efficacy of the enzyme (ALP). Which shows that there is a more effective effect of the water extract of Amygdaline on vitamin (C) when given before the carbon tetrachloride (induced) and not after (induced) for a period of 14 days, indicating that the cells were containing an antioxidant and vitamin C activator before induction is given. Although Amygdaline contains an anti-oxidant Amygdaline although vitamin C is a water soluble vitamin, Vitamin C is found in blood plasma, white blood cells and various body tissues and acts as an antioxidant, indicating its protective function of free radicals, its importance to maintain vitamin E in its effective antioxidant form (17).

Figure (7) shows the effect of the extracted dosage of Amygdaline on the efficacy of vitamin C for 14 days before and after the CCl₄ (induced). .

A = negative control (distilled water).
B = positive control CCl₄ dose of 3.2 mg / kg.
C = extract with a dose of 100 mg / kg.
D = extract with dose (200 mg / kg).
E = extract with a dose of 300 mg / kg.

**Vitamin E**

The results of this study for the effect of three different concentrations of Amygdaline extract on the vitamin E in the blood of the laboratory mice before and after the 14-day dose of the CCL₄ effect during the duration of the dosage compared with (8). This study observed a decrease in the level of (vitamin E) in the treated group (100 mg / kg) of the water extract of Amygdaline compound compared to With the treated group CCl₄ (induced), either in the treatment group (200 mg / kg) (100 mg / kg) was found to be less than 100 mg / kg, which was found to be 300 mg / kg higher than 100 mg and 200 mg / kg, (Amygdaline) on activity and efficacy on vitamin E. This indicates that there is a more effective effect of the water extract of Amygdaline on vitamin E when given prior to carbon tetrachloride (induced) and not yet induced for a period of 14 days.
indicating that the cells were containing an antioxidant and activated to Vitamin (E) before giving induced. The Amygdaline contains effective compounds that have the potential to avoid any damage. This enhances the role of the anti-oxidant Amygdaline. Vitamin E is one of the fat-soluble antioxidants and works to protect the hormones in cellular membranes from oxidative damage and vitamin E has an effect on the cellular immune response (18).

Figure (8) illustrates the effect of the extract of the Amygdaline on the efficacy of vitamin E for 14 days before and after the CCl₄ (induced) compound.

A = negative control (distilled water).
B = positive control CCl₄ dose of 3.2 mg / kg.
C = extract with a dose of 100 mg / kg.
D = extract with dose (200 mg / kg).
E = extract with a dose of 300 mg / kg.

Conclusions:
The results of this study show that the high concentration of water extract of amygdaline has high value benefits. It is recommended to avoid the negative health effects caused by contaminants, including carbon tetrachloride because it contains flavonoids as well as essential substances to control the efficacy of ALP, (GST) as well as non-enzymatic antioxidants (vitamins A, C, E) in animal laboratory serum before and after the induction of carbon tetrachloride (19).

References
1- F. Gunstone; “Specialty oils of vegetable origin ‘; Ind. Food Ingrid.; (3),51-54,(1994).
2. Lv.Wei-Feng,D.Ming – Yu,Z.Rui,’ Isolation and Quantization of Amygdaline in Apricot-Kernel and prunes by HPLC with solid –phase extraction , J. of Chromat,Scie.vol43,59,63,(2007).
3-k.Ja-Yong,H.Eun-Young,C.Sonhac,L.He-Hyunand I.,Yong-Moon Quantitative determination of amygdaline primers from armeniacae semen by liquid chromatography ; Journal of Chromatographic ; (814) 69-73,(2005).
4. B. Mahro., and M.Timm, Potential of biowaste from the food industry as a biomass resource. Engineering in Life Science , 7(5): 457-468,(2007)
5. S.v. Singh, G. Creadon, M. Das, H. Mukhtar, and Y.C. Awasthi, "Glutathione – S- transferases of mouse lung selective binding of benzo(a)pyrene metaboliter by the subunit which are preferentially by t- butylated hydroxyanisole"; Biochem. J.; (243), 351- 348(1987).
6. G.B. Saha, ’ Fundamentals of Nuclear Pharmacy”; 5th ed. NewYork: Springer-Verlag, (2005).
7. A .Perkins, M. Frier; Nuclear Medicine Pharmaceutical Research.1th ed. London: Taylor & Francis;178, (1999).
8. Liang, H., Yuan, QP., Dong, HR. and Liu, YM, Determination of Amygdaline in apricot seeds and cabbage by high – performance liquid chromatography. Journal of Food Composition and Analysis, 19 (5): 473-476, (2006).

9. P.R.N. Kind, and E.J King,. Essential of medical microbiology, botanical Museum, an ethnobotanical study. J.Clin. path, 7:322,326,(1984).

10. S. Reitman, and S.Franker, A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases Amer. J. Clin. Path.18: 6b,(1957).

11. C.Francoise, M. Pierre,. R.J aquiline, and J. Henri, plant extracts to accelerate healing and reduce inflammation cosmetics and toiletries. chemical clinical, Acta, 209-217(1989).

12. B.A.Khan, , A Abraham , and S. Leclanma,. Hematological and histological studies after curry leaf (Murray a Koenig I) and mustered (Brasses jounce ) feeding in rats, Indian, J. Med. Res., 102: 184-186,(2003).

13. Shi. John, Yu Jianmei, , and at el.. Food Agriculture and environmentvol. 1(2) : 42-47,(2005).

14. B.A.Khan, , A.Abraham , and S. Leclanma. Hematological and histological studies after curry leaf (Murray a Koenig I) and mustered (Brasses jounce ) feeding in rats, Indian, J. Med. Res., 102: 187-189, (2013).

15. Am Bones and JT Rossiter. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry, 67(11) : 1055-1067,(2016).

16. K Mure,. and T.G Rossman. Reduction of spontaneous mutagenesis in mismatch repair – deficient and proficient cells by dietary antioxidants. Mut. Res. Fund. and Mol. Mechanisms Mutagenesis, 480: 89-98,(2016).

17. D.A Hughes .Dietary carotenoids and immune function . Nutrition (3) : 823-827, (2017).

18. N.M Lyons., J. A Woods,. and N.M. Obrien . Alfa -Tocopherol but not gama- tocopherol inhibits, 7-beta- hydroxyl cholesterol induced apoptosis in human U937 cell. FreeRad. Res., 5: 339-349,(2017).

19. N. R. Madamanchi, and M. S. Runge, "Mitochondrial dysfunction in atherosclerosis," Circul. Res., 100(4), 460,473(2017).