



# ***Brassica Oleracea* Alcoholic Extract on Vitamin D3 Level and Some Blood Biochemical Parameters in Albino Male Rats Treated with Paracetamol**

Wafaa Mohammed Ridha Abdel Rasoul<sup>1\*</sup>, Dr. Saher Mahmood Jwad<sup>2</sup>

## **Abstract**

This work was carried out in labs of University of Kufa, beginning 1/10/2021 to 1/12/2021, and was conducted by following up on (28) albino male rats of the Sprague Dawley strain at the age of three months to determine the efficiency of the alcoholic extract of *Brassica oleracea* flowers in the prevention of the paracetamol-induced hepatotoxicity and nephrotoxicity, the weights of rats ranged between 216-246, and then alienated into four assembly s for every assembly of 7 rats, where the first assembly was a control orally dosed with tap water, and the second assembly was salted with paracetamol concentration 2000 mg / kg orally, either the third assembly was certain the alcoholic extract of *Brassica oleracea* flowers at a concentration of 500 mg/kg and the drug was certain at a concentration of 2000 mg/kg orally, while the last assembly was administrated with the alcoholic extract only through the mouth. Furthermore, the treatment continued for a period of (60) days, in the morning (at half past eight), once a day. The study included evaluation of biochemical parameters such as flats of vitamin D3, calcium, phosphate, creatinine, urea. **The conclusion** is the alcoholic extract of broccoli flowers has shown a protective role against the pathological effects of the drug, and this may be explained by its active chemical antioxidant components.

666

**Key Words:** Alcoholic Extract of *Brassica Oleracea* Flowers, Paracetamol, D3, Biochemical Parameters.

**DOI Number:** 10.14704/nq.2022.20.5.NQ22222

**NeuroQuantology 2022; 20(5):666-671**

## **Introduction**

Medicinal plants are of importance to human life for their effective role in detoxification and are associated with the absence of side effects or their reduction, and a number of medicinal plants showed a protective effect for the liver and kidneys in liver and kidney disorders caused by gentamicin, paracetamol, profenofos, toxic drugs, nephropathy, chronic pressure, diabetes and nephrolithiasis caused by oxidative stress and inflammatory mediators [1]. Broccoli is *Brassica olerace* var. Italica is one of the types of medicinal plants available in the Mediterranean region, it is an annual herbaceous plant that looks similar to cauliflower in appearance. It is characterized by its high nutritional value, it is considered one of the

foods free of sodium, fat and calories, and it is a good source of a number of vitamins such as vitamin A, C and D, riboflavin, niacin, folic acid and carotenoids, also have excellent therapeutic properties, as it is an effective a number of, as it works to reduce as well as helps lower cholesterol flat in the blood, regulate sugar flat, escalation body strength and build bones, it also contributes to protecting against heart and urinary diseases and reduces the risk of cancer, in addition it is a rich source of anti-cancer glucosinolates, and it has been proven that eating more than one meal per week reduces the risk of cancer by 45%, furthermore it contributes to preventing retinal diseases [2, 3].

**Corresponding author:** Wafaa Mohammed Ridha Abdel Rasoul

**Address:** <sup>1,2</sup>Biology Department, Faculty of Education for Women, Kufa Unive., Iraq.

**Relevant conflicts of interest/financial disclosures:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Received:** 25 March 2022 **Accepted:** 29 April 2022



Paracetamol or acetaminophen is widely used in many cases that do not require a prescription because of its, it is taken orally to relieve pain in mild, moderate and severe cases, as well as intravenous administration has also become widely spread, and the recommended therapeutic doses of the drug are suitable and 50-75 mg / kg / day for children, and when consuming doses more than 7 g in adults and 150 mg / kg in children, it is toxic to the liver and kidneys because of the toxic compound N-acetyl-para-aminophenol (NAPQI) that is highly effective formed by the oxidation of the drug through the cytochrome pathway, it has many functions in metabolism and ion balance, and it is of several types, the most common types of vitamin are D3 and D2, as well as they are converted into the biologically active form, which contributes to the transport of phosphate and calcium in addition to other activities [4], vitamin D has recently received great interest in the treatment of strokes cerebral palsy, heart disease and mental disorders [5].

### Experimentations

A assembly of (28) animals in our work applied, their weights ranged between 216-246 g and their age was less than 3 months (did not reach sexual maturity) then the laboratory animals entered into lab and were salted with good care by providing the appropriate laboratory conditions for it, and certain water and animal ration designated for it, rich in protein, in appropriate quantities until it became sexually mature, after which it was subjected to the experiment.

#### Preparation of Paracetamol Drug

The drug was obtained from the pharmacy / Najaf Al-Ashraf with a (2000 mg per kg), and the original solution was prepared [6], and certain to each rat according to its weight.

#### Stock Solution Preparation Broccoli Plant Extract

The dry matter of the crude alcoholic extract was dissolved at 500 mg per kg in 1 ml of distilled water to prepare the original solution and the dose was certain depending on the weight of the animal, noting that the half-lethal dose (LD50) for broccoli is greater than 21 g / kg body weight [7].

#### The Study Assembly s

- The control assembly contained 7 rats, and they were certain tap water for 60 consecutive days.

- The drug assembly included 7 rats orally dosed with paracetamol at a concentration of 2000 mg/kg once, for 60 in sequence times.
- The third assembly, the drug and extract assembly, included 7 rats who were first certain the alcoholic extract of *Brassica oleracea* flowers 500 mg/kg orally, and then the drug once a day for 60 consecutive days.
- The fourth assembly contained 7 rats that were salted with the extract only and once a day for 60 days without interruption.

#### Sacrifice of Experimental Animals

After completing our work, the laboratory rates were sedated with xylazine and ketamine, then the blood stayed drawn by heart, and 5 ml of blood was placed in a gel tube for determination of biochemical parameters of blood.

#### A. Estimation - Flat of Vitamin D3 in the Serum

The assay kit used by (Elab science) was prepared to evaluate the flat of vitamin D in serum samples by ELISA technique, and the absorbance was read at the wavelength 450nm.

#### B. Estimation of Calcium Ion Flat in Serum

Serum calcium was determined according to the method [8], with COBAS C311 from Roche.

#### C. Estimation of Phosphate Ion Flat in Serum

The serum phosphate flat was evaluated by using the method [9], and COBAS C311 from Roche/Hitachi.

#### D. Estimation of AST Activity in Serum

Evaluation of AST enzyme activity according to the method [10], the kit via the Syrian, the measurement was calculated at the wavelength of 546 nm.

#### E. Estimation of (ALT)

Rendering to the method [10] for (ALT), chemical reagents and the test kit supplied by the Syrian company (Syrbio) were evaluated, as well as the absorbance reading at the wavelength of 546 nm.

#### F. Estimation of ALP Enzyme Activity in Serum

Alkaline phosphatase ALP was evaluated using the ready-made kit from the French company (Biomerieux) and the method was adopted [11],



and the enzyme activity was estimated at the wavelength 510 nm.

**G. Estimation of Serum Creatinine Flat**

The method was adopted [12] to evaluate the flat of creatinine in the serum, as all reagents and equipment were prepared from (Biolabo) company / France, in addition to reading the absorbance at the wavelength 490 nm.

**H. Estimation of Serum Urea Flat**

The diagnostic kit was equipped from the company (Biomerieux) / France to assess the flat of urea in the serum as stated in [12], in addition to that reading the absorbance at 580 nm.

**I. The Statistical Analysis**

With regard to the analysis of the results of this study, the statistical program (SPSS) and the data analysis test (ANOVA) were used, all treatments depending on the LSD value at probability (P<0.05) [13].

**Results**

This work showed a momentous decrease (P<0.05) in the flats of vitamin D3, calcium and phosphate in the assembly that was salted with paracetamol paralleled to the control assembly and the rest of the assembly s. In contrast, the assembly that was certain the alcoholic extract of *Brassica oleracea* flowers recorded a momentous escalation (P<0.05) in the flat of vitamin D3, calcium and phosphate ions in paralleled to the control and other assembly s, while no momentous differences (P>0.05) were recorded in those parameters when comparing the rest of the assembly s with each other, as shown in table (1).

**Table 1.** Effect of treatment with alcoholic extract of *Brassica oleracea* flowers and paracetamol on the flats of vitamin D3, calcium and phosphate ions

Assembly s of study	Number of samples	Mean ±SE		
		D3 pg/ml	Ca mg/dl	Po <sub>4</sub> mg/dl
G1	7	280.29 ±0.808 b	9.37 ±0.087 b	3.94 ±0.104 b
G2	7	191.86±2.086 c	6.53±0.108 c	2.08±0.056 c
G3	7	279.56±0.841 b	9.31±0.05 b	3.84±0.085 b
G4	7	295.57±1.96 a	9.92±0.05 a	4.27±0.091 a
LSD (0.05)		4.512	0.2259	0.250

This study revealed a remarkable escalation (P<0.05) in the commotion of hepatic enzymes in the serum of the assembly that was certain paracetamol paralleled with the other experimental assembly s, but no momentous differences (P>0.05) were recorded in the activity of those enzymes when comparing the rest of the study assembly s with each other, as shown in table (2).

**Table 2.** *Brassica oleracea* flowers and paracetamol on the commotion of liver in serum

Assembly	samples	Mean±SE		
		AST Unit per liter	ALT unit per liter	ALP Unit per liter
G1	7	38.00 ±0.78 b	48.86 ±0.77 b	148.14 ±0.86 b
G2	7	89.71±1.29 a	120.43±0.65 a	289.57±2.26 a
G3	7	38.86±1.01 b	49.71±0.78 b	149.28±1.41 b
G4	7	38.28±1.87 b	47.57±0.19 b	147.43±1.45 b
LSD (0.05)		3.846	2.545	4.591

The findings flats of creatinine and urea in the serum of rats dosed with paracetamol paralleled with the other assembly s in the study, but no momentous fluctuations (P>0.05) in the flats of those parameters when comparing the other assembly s with each other, as shown in table (3).

**Table 3.** Effect of alcoholic extract of *Brassica oleracea* and paracetamol on the flats of creatinine and urea

Assembly	samples	Means± SE	
		Creatinine Mg-dl	Urea mg-dl
G1	7	0.204 ±0.024 b	21.29 ±0.68 b
G2	7	2.26± 0.986 a	55.00±1.54 a
G3	7	0.237±0.02 b	22.43±0.95 b
G4	7	0.210±0.02 b	21.43±0.84 b
LSD (0.05)		0.0671	3.078

**Discussion**

Our present work revealed a momentous decline in flats of vitamin D3, calcium and phosphate ions in the assembly salted with paracetamol in comparison to the control assembly and the rest of



the experimental assembly s. The reason may be attributed to the hepatic damage caused by the drug to the liver as a result of the formation of free radicals, oxidative stress and depletion of glutathione due to the escalation in the generation of the toxic reactive compound of the drug and the stimulation of programmed cell death [14], because the first step in which vitamin D is converted to 25(OH)D occurs in the liver by the enzyme 25-hydroxylase [15].

Furthermore, this may be explained by the renal failure caused by the drug as a result of severe tubular necrosis in the kidneys, abnormalities of the proximal convoluted tubules, the emergence of edema and degeneration of the epithelial layer lining, as documented during the current study, in addition to a number of other pathological changes that were diagnosed in the histological sections of the kidneys, because the second step the conversion of 25(OH)D to its active form occurs in the kidneys by the enzyme 1 $\alpha$ -hydroxylase present in the renal tubular epithelial cells, and therefore, any pathological alterations in the renal tissues may negatively affect the metabolism of vitamin D through a decrease in the activity of  $\alpha$ -hydroxylase 1, and the loss of the ability of the kidneys to subsequently generate the active form of the vitamin, 1,25 (OH)<sub>2</sub>D and a decrease in its serum flats as well as the content of its receptors in tissues [16].

Moreover an elevation in the parathyroid hormone and a disturbance in the balance of calcium and phosphate ions, then damage to the kidneys and blood vessels [17], as vitamin D3 inhibits the production of parathyroid hormones as part of the feedback, which leads to a decrease in calcium absorption through the intestines and kidneys, in addition an escalation in its excretion in urine, and stimulates the production of fibroblast growth factor by osteoblasts to legalize the steadiness of Ca and PO<sub>4</sub> ions, which results in a rise in phosphate excretion in urine [15].

As for the assembly that dosed with the alcoholic extract only, it recorded a noticeable escalation in the flats of vitamin, calcium and phosphate when paralleled with the resistor assembly and the rest of the experimental assembly s within this study. This may be explained by the protective role of the extract due to the highly effective minerals, vitamins and phytonutrients it contains in protecting the various tissues of the body [18].

The data of the current study indicated to an escalation in the inefficiency in enzymes AST, which

was similar to some studies [19]. The reason likely to be due to the drug has caused liver dysfunction and reduced the activities of antioxidants, which led to the liberation of free radicals, oxidative stress and subsequent increased flats of malondialdehyde, which caused an escalation in the permeability of hepatocytes and the exit of these enzymes as well as their entry into the bloodstream, as proved by some studies [20].

In addition to the high flats of the reactive metabolite NAPQI formed by the metabolism of paracetamol at high doses of it [21], which causes the consumption of 90% of glutathione in hepatocytes and mitochondria, that caused mitochondrial dysfunction, DNA damage, programmed cell death and hepatic necrosis, which led to increased cellular membrane permeability and cell rupture [22]. The depletion of glutathione results in an elevation in the generation of free radicals and thus an escalation in the flat of oxidative stress [23], and the activity of liver enzymes.

Concern to the assembly that was salted with the alcoholic extract only, no momentous differences were recorded in the effectiveness of hepatic enzymes paralleled with the other assembly s of the trial. This may be suggested to the preventive role of antioxidants and the active chemical constituents that the extract possesses, as phenol is one of the powerful antioxidants, it participates in redox reactions by inhibiting free radicals liberation and thus reducing oxidative stress [24], hence maintaining the effectiveness of hepatic enzymes by protecting cellular proteins and preventing hepatocyte death.

The results of this study showed a notable escalation in creatinine and urea flats in male rats dosed with paracetamol, and it was in agreement with some studies [25]. The reason may be due to severe tubular necrosis caused by the drug, which is one of the main causes of kidney failure, as confirmed by some studies [26]. Moreover, the high flats of urea and creatinine may explain the functional decline of the glomeruli, which is affected in filtration rates due to the oxidative stress resulting from the drug, most studies have shown that paracetamol stimulates the generation of free radicals, which is one of the main causes of acute renal failure, in addition to the oxidation of fats in cell membranes, and thus glomerular shrinkage, this leads to a reduction in the surface area of the filtration, which negatively affects the filtration rates [27].



Furthermore, renal insufficiency results in a decrease in the activity of vital nephrons, and then the accumulation of metabolic wastes such as urea and creatinine in the serum, a decline in their filtration rates, and a reduction for body fluids due to the glomerular filtration failure, as indicated by some studies [28].

With regard to the alcoholic source assembly, it disappeared any momentous differences in the flats of urea and creatinine when paralleled with the other assembly s, and this may be due to the absence of any pathological effects in the tissue sections of the kidneys, as the glomeruli of the kidneys were observed intact with the epithelium lining them, the nucleus and the cytoplasm, as well as no atrophy or shrinkage of the glomeruli or congestion or hemorrhage inside it, and the epithelium lining the convoluted renal tubules did not appear any noticeable necrosis or degeneration [29, 30]. As for the interstitial tissue of the renal cortex, it did not suffer from hemorrhage or edema; this may be attributed to the active physiological role of broccoli for its effective elements that improve the functional performance of the renal tissues.

## References

- Nazir, N.; Muhammad, J.; Ghaffar, R.; Nisar, M.; Zahoor, M.; Uddin F. and Alotaibi, A. (2021). Phytochemical profiling and antioxidant potential of *Daphne mucronata* Royle and action against paracetamol-induced hepatotoxicity and nephrotoxicity in rabbits. *Saudi J. Boil. sci.*, 28(9): 5290-5301.
- Wafaa M.R, Jwad S.M. Evaluation of the efficacy of *Brassica oleracea* L. alcoholic extract on the flats of D3 and antioxidants in albino male rats dosed with Paracetamol, *Uttar Pradesh Journal of Zoology*, 2022, 43(6): 72-77.
- Al-Hussainy, E.J. and Manea, A.I. (2019). Effect of planting distance and organic fertilization on growth and yield of broccoli (*Brassica oleracea* var. *Italica*). *Euphrates J. Agricul. Sci.*, 11(4): 13-21.
- Mazaleuskaya, L.L.; Sangkuhl, K., Thorn, C.F.; FitzGerald, G.A.; Altman, R.B. and Klein, T.E. (2015). Pharm GKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenetics. Genomics.*, 25(8): 416.
- Charoenngam, N.; Shirvani, A. and Holick, M.F. (2019). Vitamin D for skeletal and non-skeletal health: What we should know. *J. Clin. Orthopaedics. Trauma.*, 10(6): 1082-1093.
- Bansal, C. J and Bansal, A.S. (2019). Stress, pseudoallergens, autoimmunity, infection and inflammation in chronic spontaneous urticaria. *Allergy. Asthma. Clin. Immunol.*, 15(1): 1-11.
- Sounder, L.; Doss, V.A. and Mohanasundaram, S. (2018). Acetaminophen induced nephrotoxicity in experimental albino rats- histopathological study. *Int. J. Res. Pharm. Sci.*, 9(3): 907-910.
- Geetha, T.S and Geetha N. (2014). Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogon citratus* (DC) stapf. Leaves from Kodaikanal hills, Tamilnadu, *International J. Pharm. Tech. Res.*, 6(2): 521- 529.
- Shipping, P.; Company, C. and Entry, D. (2006). Standard Operating Procedure : 10(1): 56-57.
- Weissman, M.; Pileggi, V.J.; Henry, R.J.; Cannon, D.C. and Winkelman, J.W. (1974). *Clinical Chemistry: Principles and Techniques*. 11th Edition, Harper and Row Publishers, Hagerstown, MD:655.
- Huang, X.J.; Choi, Y.K.; Im, H.S.; Yarimaga, O.; Yoon, E. and Kim, H.S. (2006). Aspartate aminotransferase (AST/GOT) an alanine aminotransferase (ALT/GPT) detection techniques. *Sensors (Basel).*, 6(7) : 756-782.
- Tietz, N.W. (1999). *Text book of clinical chemistry*. 3rd ed. C.A. Burtis E.R. ashwood, W.B. Saunders, 282-884 and 1429-1431.
- Wopara, I.; Uwakwe, A.A.; MODO, E.U.; Ikenazor, H.O.; Offor, H. and Ofodile, J.O. (2018). Possible effect of *Sphenocentrum jollyanum*, *Baphia nitida* and seeds of *Pinus koraiensis* and *sildenafil* sodium, potassium, urea and creatinine flats of albino rats. *Int. J. Biol. Res.*, 3(2): 45-50.
- Morgan, G.; Leech, N.A.; Gloecner, G.W. and Barrett, K.C. (2010). *SPSS for introductory statistic: use and interpretation*. 2nd ed. Lawerenz Erlbum associatiates, publisers Mahwah, New Jersey Lonbon.
- Abood, A.M.; Awad, H.; E.; A. and Hassan, S.M. (2021). Potential role of vitamin D receptor agonist in acetaminophen-induced hepatic injury: role of caspase-1. *Ain. Shams. Med. J.*, 72(2): 251-265.
- Charoenngam, N.; Shirvani, A. and Holick, M.F. (2019). Vitamin D for skeletal and non-skeletal health: What we should know. *J. Clin. Orthopaedics. Trauma.*, 10(6): 1082-1093.
- Dusso, A.S., and Tokumoto, M. (2011). Defective renal maintenance of the vitamin D endocrine system impairs vitamin D renoprotection: a downward spiral in kidney disease. *Kid. Int.*, 79(7): 715-729.
- Uitterlinden, A.G.; Fang, Y.; Van Meurs, J.B.J.; Pols, H.A.P. and van Leeuwen, J.P.T.M. (2004). Genetics and biology of vitamin D receptor polymorphisms. *Gene.*, 338(2): 143-156.
- Bongoni, R.; Verkerk, R.; Steenbekkers, B.; Dekker, M. and Stieger, M. (2014). Evaluation of different cooking conditions on broccoli (*Brassica oleracea* var. *italica*) to improve the nutritional value and consumer acceptance. *Plant. Foods Human. Nutr.*, 69(3): 228
- Hamza, R.Z. and Al-Harbi, M.S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and *Nigella sativa* extract supplements. *Asian Pacific J. Tropical. Biomed.*, 5(7): 521-531.
- Mossa, A.T.H.; Heikal, T.M. and Omara, E.A.A. (2012). Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. *Asian Pacific J. Tropical. Biomed.*, 2(3): S1683-S1690..
- Abdul-Zahra, M.T., Jwad, S.M. (2020). The Effect of Alcoholic Extract of Pomegranate Peel on the flat of Erythropoietin and some of the Blood Properties in Albino Male Rats salted with Erythromycin. *International Journal of Psychosocial Rehabilitation*, 24(04), 6350-6361.
- Andersson, D.A.; Gentry, C.; Alenmyr, L.; Killander, D., Lewis, S.E.; Andersson, A. and Zygmunt, P.M. (2011). TRPA1



- mediates spinal antinociception induced by acetaminophen and the cannabinoid  $\Delta^9$ -tetrahydrocannabinol. *Nature Communications*, 2(1): 1-11.
- Walle, L.; V. and Lamkanfi, M. (2016). Pyroptosis. *Current Biol*, 26(13): R568-R572.
- Hinson, J.A.; Roberts, D.W. and James, L.P. (2010). Mechanisms of acetaminophen-induced liver necrosis. *Adverse. Drug. reactions*, 196: 369-405.
- Köksal, E., and Gülçin, İ. (2008). Antioxidant activity of cauliflower (*Brassica oleracea* L.). *Turkish J. Agricult. Forestry*, 32(1): 65-78.
- Canayakin, D.; Bayir, Y.; Baygutalp, N.K.; Karaoglan, E.S.; Atmaca, H.T.; Ozgeris, F.B.K.; Keles, M.S. and Halici, Z. (2016). Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of *Nigella sativa*. *Pharm. Biol*, 54(10): 2082–2091.
- Zahraa Sami Razzaq Najjar, Saher Mahmood Jwad, Rafie Shakir Alkhafaji. (2020). Evaluation of VDR Gene Polymorphisms with Nephropathy Stages in Men with Type 2 Diabetes Mellitus. *J. Cardiovasc Disease Res*, 2020; 11(4): 275-279, DOI: 10.31838/jcdr.2020.11.04.49
- Öksüz, E.; Buğday, M. S.; Demir, F.; Ekin, S.; Arıhan, O. and Oto, G. (2020). Serum total sialic acid and lipid-linked sialic acid may be the new potential biomarkers in paracetamol nephrotoxicity. *Turkish. J. Nephrol*, (Online), 29(4): 273-278.
- Hegazy, A.; Abd Al Hameed, E.A.; El-Wafaey, D. and Khorshed, O. (2021). Effect of paracetamol administration on the rat kidney structure: A Morphological Study. *Zagazig University Med. J*, 27(4), 567-576.
- Guyton, A.C. and Hall, J.E.; (2006). *Textbook of physiology*. 11th ed., W.B. Saunders Co. Philadelphia, U.S.A., 961-974.