**UTTAR PRADESH JOURNAL OF ZOOLOGY**

43(6): 72-77, 2022
*ISSN: 0256-971X (P)*

# EVALUATION OF THE EFFICACY OF *Brassica oleracea* L. ALCOHOLIC EXTRACT ON THE LEVELS OF D3 AND ANTIOXIDANTS IN ALBINO MALE RATS DOSED WITH PARACETAMOL

**WAFAA MOHAMMED RIDHA ABDEL RASOUL [a≡] AND SAHER MAHMOOD JWAD [a*ϖ]**

[a] Department of Biology, Faculty of Education for Girls, Kufa University, Iraq.

**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration between both authors. Authors WMRAR, SMJ managed the research. Authors WMRAR and SMJ done the research and wrote the main manuscript text. Authors WMRA, SMJ prepared tables and wrote a part of manuscript text. Both authors read and approved the final manuscript.

***Article Information***

DOI: 10.56557/UPJOZ/2022/v43i62984

*Editor(s):*
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
*Reviewers:*
(1) Subhash R. Somkuwar, RTM Nagpur University, India.
(2) Ademu Haruna, Federal university of Technology Minna, Nigeria.
(3) Arun Kumar Ramu, India.

***Received: 13 February 2022***
***Accepted: 26 April 2022***
***Published: 29 April 2022***

***Original Research Article***

## ABSTRACT

**The Aim:** The study included assessment of biochemical parameters such as levels of vitamin D3, reduced and oxidized glutathione, malondialdehyde and lipid peroxide.
**Place and Duration of Study:** This study was carried out in the animal house of the Faculty of Education for Girls / University of Kufa, from 1/10/2021 to 1/12/2021.
**Study Design:** as twenty-eight male albino rats of the Sprague Dawley strain were followed at the age of three months to evaluate the protective efficiency of the alcoholic extract of the *Brassica oleracea* flowers against paracetamol-induced hepatotoxicity and nephrotoxicity.
**Methodology:** Rats' weights were between 216-246, divided into 4 groups in each group, 7 rats. The first group was dosed orally with tap water, which is the control group, and the second group was treated with paracetamol at a concentration of 2000 mg / kg orally, the third group was administrated with the alcoholic extract of *Brassica oleracea* flowers at a concentration of 500 mg/kg + paracetamol at a concentration of 2000 mg / kg orally as well, and with regard to the last group, only the alcoholic extract was given, where the dosing process lasted for 60 days ,once a day.
**Results :** The study included assessment of biochemical parameters such as levels of vitamin D3, reduced and oxidized glutathione, malondialdehyde and lipid peroxide. The results of the statistical analysis recorded a

[≡] *Student;*
[ϖ] *Professor;*
**Corresponding author: Email: sahermahmood45@gmail.com;*

significant (P<0.05) decrement in the levels of vitamin D3, reduced and oxidized glutathione, in contrast, a notable (P<0.05) increase in the levels of malondialdehyde and lipid peroxide in the group that was submitted to paracetamol when compared with the control group and the rest of the experimental groups. As for the group that was subjected to the extract only, it showed a significant (P<0.05) increment in the levels of vitamin D3, reduced and oxidized glutathione, and conversely a remarkable decrease (P<0.05) in the levels of malondialdehyde and lipid peroxide compared with the control group.

**Conclusion:** The conclusion is that the alcoholic extract of *Brassica oleracea* flowers has shown a preventive activity against the pathological effects of paracetamol, possibly due to its chemical antioxidant contents.

**Keywords:** Alcoholic extract of *Brassica oleracea* flowers; paracetamol; D3; biochemical parameters.

## 1. INTRODUCTION

Broccoli is a fast-growing plant with a length of 60-90 cm and its origins back to the eastern Mediterranean and Asia Minor, *Brassica oleracea* belongs to the family Brassicaceae, and was later introduced to England and America in the eighteenth century AD [1], and is considered an important medicinal plant due to it contains many antibacterial factors [2], and broccoli also provides numerous of the properties necessary for health that are attributed to its antioxidant compounds and consists mainly of selenium, glucosinolates and sulforaphane [3].It has a major role in the decomposition of some compounds in particular indole-3 carbinol, which has the ability to stimulate the formation of tumors, and therefore small amounts of it may protect against the risk of cancer and with the same effectiveness as larger quantities of vegetables of the same type, and also contains a large proportion of compounds such as flavonoids, mineral nutrients and vitamins especially vitamin C, which is also a good helper in the treatment of iron deficiency, and can interfere with the metabolism of some drugs ,as well as antitumor agents, in addition to the presence of such contents made broccoli vital in protecting humans from certain types of cancer, and one of the therapeutic benefits of broccoli for skin diseases, as its juice is used to treat warts [1]. Manufactured paracetamol is chemically known as N-acetyl-para-aminophenol (APAP) as a non-steroidal anti-inflammatory drug (NSAID), for the first time in 1878 by Morcy, and first used clinically by von Mering in 1887, paracetamol does not cause gastrointestinal damage or undesirable cardiac and renal effects if the recommended therapeutic doses are used [4]. The drug is one of the most widespread and popular antipyretic drugs, as well as analgesics in the world [5], besides one recent study showed that a high dose of it could lead to acute liver necrosis in humans and experimental animals.

Furthermore it has been proven that the unhealthy use of paracetamol may cause hepatotoxicity especially for the elderly, children , liver patients, , cases of alcoholism and patients suffering from chronic malnutrition, as well as poisoning may be fatal in these cases, moreover high doses of it may lead to acute renal tubular necrosis, and symptoms appear during the first 24 hours including vomiting, nausea , abdominal pain ,loss of appetite, pallor, hemolytic hepatitis and complete necrosis of the liver, which causes acute liver failure, insufficiency in the work of liver cells and encephalopathy that ends in coma and death [6].

Vitamin D exists in two forms:D3 (Chlecalciferol), which is the most important source in animals and produced in the skin when exposed to sunlight, and vitamin D2 (Ergocalciferol), which is created from ergosterol found in mushrooms, yeast, phytoplankton, the sun and ultraviolet rays, which differs from vitamin D3 contains a methyl group in C24 and a double bond between C22-C23 [7]. After the body obtains vitamin D from sunlight and food, vitamin D is converted in the liver to 25 (OH) D by the enzyme 25- hydroxylase, and then is conversion of inactive vitamin D 25(OH)D to the active form1, 25(OH)2D in the kidneys by the enzyme 1 α-hydroxylase, which belongs to the cytochrome family p450 (CYP27B1) [8].

## 2. MATERIALS AND METHODS

### 2.1 Preparation the Experimental Animals

In the current study, a group of 28 sexually adult male albino rats were used, and their weights ranged between 216-246 g, which was obtained from the animal house belonging to the College of Science / University of Kufa, and then entered the animal house of the College of Education for Girls / University of Kufa, and was carefully treated according to the directives of the Ethics Committee of the University of Kufa, as appropriate laboratory conditions were provided for it and given water, as well as animal feed allocated to it, rich in protein in sufficient quantities until completion of the experiment.

### 2.2 Preparation of Paracetamol Drug

The drug was taken from the pharmacy / Najaf Al-Ashraf with a dose of 2000 mg / kg, then the original

solution was prepared [9], and it was given to the rat according to its weight.

## 2.3 Stock Solution Preparation of Broccoli Plant Extract

The dry matter of the crude extract was dissolved at a concentration of 500 mg / kg in 1 ml of distilled water to prepare the original solution (Stock solution) and the dose was given depending on the weight of the animal, knowing that the lethal dose fifty (LD50) for broccoli is greater than 12 g / kg body weight [10].

## 2.4 The Study Groups

- The first group included 7 rats, which were dosed with tap water for 60 days, which is the control group.
- The second group contained 7 rats that were orally given paracetamol once, and this lasted for 60 continuous days, at a rate of 2000 mg/kg, which is the drug group.
- The third group consisted of 7 rats that were orally administrated with the alcoholic extract of broccoli flowers 500 mg/kg first, and an hour after the first dose they were treated orally with paracetamol 2000 mg/kg once daily for a continuous period of 60 days, which is the drug and extract group.
- The fourth group, which included 7 rats, was treated orally with the extract only once a day for a 60 consecutive days, which is the extract group.

## 2.5 Sacrifice of Experimental Animals and Collection of Blood Samples

After completing the experiment, the animals were anesthetized by xylazine and ketamine, and blood samples were collected for each animal directly from the heart. The sample amount was (5 ml) placed in a gel tube to evaluate the biochemical parameters.

## 2.6 The Biochemical Evaluation of Blood

### A. Estimation of the level of vitamin D3 in the serum

An assay kit supplied by (Elab Science) was used to assess the level of vitamin D in serum samples by ELISA technique, and at wavelength 450 nm the absorbance was calculated.

### B. Estimation of GR level in the serum

The method [11] was adopted to assess the level of reduced glutathione in the serum by the enzymatic cycling method, after which the absorbance was measured at wavelength between 405-414nm in an ELISA device.

### C. Estimation of GPx1 level in the serum

The level of oxidized glutathione in the serum was assessed by using the assay kit supplied by BTLAB company and the ELISA device according to method [12], and its absorbance was estimated at a wavelength of 412 nm.

### D. Estimation of MDA level in the serum

The level of malondialdehyde was evaluated according to the method [13], and the absorbance was recorded at the wavelength 540-530 nm.

### E. Estimation of lipid peroxide level in the serum

The level of lipid peroxide in the serum was evaluated according to the method [12], and the absorbance was read at the wavelength (450) nm.

### F. Statistical analysis of results

With regard to the analysis of the study findings, the statistical program (PSS) and the method of analysis of variance (NOVA A) were used, and significant S differences were recorded between all experimental groups, depending on LSD at the probability level ($P<0.05$) [14].

## 3. RESULTS

The results of this trial showed a significant decrease ($P<0.05$) in the levels of vitamin D3 in the group that had been dosed with the drug when compared with the control group and other study groups, and in contrast to it, the alcoholic extract group recorded an observable increase ($P<0.05$) in the level of vitamin D3 in compared to the control group and the rest of the groups. As shown in Table (1).

The results of the statistical analysis of this study revealed a significant reduction ($P<0.05$) in the levels of reduced and oxidized glutathione in the group that was treated orally with paracetamol compared with the rest of the other experimental groups, and in contrast to it a noticeable rise ($P<0.05$) in the levels of malondialdehyde and lipid peroxide in the serum comparison to the control and the other groups. Otherwise, the extract exhibited a significant elevation ($P<0.05$) in the levels of reduced and oxidized glutathione, whereas a remarkable decrease ($P<0.05$) in the levels of malondialdehyde and lipid peroxide comparing with the control group and other groups of study, as shown in Table (2).

**Table 1. Effect of treatment with alcoholic extract of *Brassica oleracea* flowers and paracetamol on the level of vitamin D3**

| Groups of study | | Mean ±SE |
|---|---|---|
| | Number of samples | D3 pg/ml |
| G1 | 7 | 280.29 ±0.808 b |
| G2 | 7 | 191.86±2.086 c |
| G3 | 7 | 279.56±0.841 b |
| G4 | 7 | 295.57±1.96 a |
| LSD (0.05) | | 4.512 |

✹ *Different letters mean significant differences between the experimental groups at P-value < 0.05*

**Table 2. Effect of treatment with alcoholic extract of broccoli flowers and paracetamol on antioxidants (reduced and oxidized glutathione) and oxidative stress parameters (malondialdehyde and lipid peroxide) in serum**

| Groups of study | Mean ±SE | | | | |
|---|---|---|---|---|---|
| | Number of samples | GSHr ng/ml | GPX1 nmol/ml | MDA nmol/l | LPO nmol/ml |
| G1 | 7 | 186.43±0.99 b | 38.04 ±0.64b | 0.919 ±0.01 b | 3.877 ±0.09 b |
| G2 | 7 | 19.574±0.65 c | 8.97±0.06 c | 1.981±0.04 a | 6.984 ±0.05 a |
| G3 | 7 | 167.00±0.82 b | 37.94±0.25 b | 0.918±0.01 b | 4.014±0.08 b |
| G4 | 7 | 273.71±3.62 a | 50.39±0.51 a | 0.493±0.009 c | 3.231±0.04 c |
| LSD (0.05) | | 5.692 | 1.249 | 0.0666 | 0.2011 |

✹ *Different letters mean significant differences between the experimental groups at P-value < 0.05*

## 4. DISCUSSION

The current study diagnosed a significant decrease in the level of vitamin D3 in the group that was dosed with the drug compared to the control group and other experimental groups. This may be explained by the hepatotoxicity caused by paracetamol to the liver tissues as a result of the generation of detrimental free radicals, oxidative stress and depletion of glutathione molecules due to the increase in the formation of the toxic intermediate compound of the drug and the stimulation of programmed cell death [15], because the first stage in which vitamin D is converted to 25(OH)D occurs in the liver by the 25-hydroxylase enzyme [8].

Furthermore, the reason may be attributed to the renal failure that may cause by the drug as a result of acute tubular necrosis in the kidneys and deformation of the proximal convoluted tubule with the appearance of edema ,infiltration, and degeneration of the epithelial lining layer as recorded in this study ,in addition to many other histological changes observed in the renal sections, because of the second stage of the conversion of 25(OH)D to the active form is carried out in the kidneys by the enzyme 1α-hydroxylase in the renal tubular epithelial cells, and therefore any pathological effects in the kidney tissues may affect the metabolism of vitamin D due to the decline in the activity of 1α-hydroxylase ,thereby the kidneys subsequently lose their ability to generate 1,25 $(OH)_2$ D, the active form of vitamin D3, and decrease its levels in the blood serum ,as well as the content of vitamin D receptors in tissues [16,17].

As for the group that was given the alcoholic extract only, it recorded a significant increase in the level of vitamin D3 compared to the control group and the experimental groups, the finding likely to be due to its high content of vitamin C, E and A ,as well as an important source of manganese, selenium and calcium, in addition, broccoli contains active chemical compounds in particular carotenoids, which include lutein and beta-carotene act as powerful antioxidants to control diabetes by reducing blood sugar level and oxidative stress. Moreover, the sulforaphane present in broccoli extract also prevents neuro-degeneration, which has a positive effect in Alzheimer's and Parkinson's patients, as well as other anti-inflammatory and oxidative stress properties [18].

The results of oxidative stress indicators in the group administrated with paracetamol recorded a reduction in the levels of reduced GSHr and oxidized GPX1 glutathione, on the other hand an increase in the levels of MDA and lipid peroxide when compared with the control group and the other study groups, which was agreement with some studies [19, 20]. The observable decline in the levels of reduced and oxidized glutathione may explain the increase in MDA production and lipid peroxidation as a result of oxidative stress, the liberation of adverse free radicals and reactive oxygen species due to high doses (2000 mg/kg) of paracetamol, which caused the depletion of

the cellular store of glutathione molecules, as glutathione GSH is a tripeptide, found in many mammalian tissues, it conjugates with the reactive component NAPQI of paracetamol and is excreted in the urine [21,22].Besides, glutathione is primarily an antioxidant system that scavenges free radicals, such as hydrogen peroxide and superoxide radicals, then maintains on membranous proteins, hence the decreased levels of GSH ,and conversely the high levels of MDA lead to drug-induced nephrotoxicity [23,24,25].

Concern to the group that was submitted to the extract only, it was observed a significant increment in the levels of reduced and oxidized glutathione, and on the contrary, a decrement in the levels of malondialdehyde and lipid peroxide compared to the control and the other groups, this possibly because of the extract possesses effective antioxidants especially vitamin C ,which prevents the hepatotoxicity and nephrotoxicity [26,27] ,moreover potent mineral compounds [28] , as they are considered vital protective factors act synergistically to inhibit or reduce the oxidative damage, by suppressing lipid peroxidation process and subsequent formation of hydrogen peroxide ,as well as stimulating the enzymatic activity of active antioxidants [29].

## 5. CONCLUSION

Is that the alcoholic extract of *Brassica oleracea* flowers has shown a preventive activity against the pathological effects of paracetamol, possibly due to its chemical antioxidant contents. The observable decline in the levels of reduced and oxidized glutathione may explain the increase in MDA production and lipid peroxidation as a result of oxidative stress, the liberation of adverse free radicals and reactive oxygen species due to high doses (2000 mg/kg) of paracetamol, which caused the depletion of the cellular store of glutathione molecules, as glutathione GSH is a tripeptide

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Saeed M, Naveed M, Leskovec J, Ali Kamboh A, Kakar I, Ullah K, Ahmad F, Sharif M, Javaid A, Rauf M, Abd El-Hack ME, Abdel-Latif MA, Chao S.Poult Sci. Using Guduchi (Tinospora cordifolia) as an Eco-friendly Feed Supplement in Human and poultry Nutrition. 2020;99(2):801-811. DOI: 10.1016/j.psj.2019.10.051. Epub 2019 Nov 29.
2. Wadood N, Wadood A, Nisar M. Effect of ficus relegiosa on blood glucose and total lipid levels of normal and alloxan diabetic rabbits. .J Ayub Med Coll Abbottabad. 2003;15(4):40-2.
3. Ravikumar C. Therapeutic potential of *Brassica oleracea* (broccoli)–a review. Int. J. Drug Dev. Res. 2015;7(2): 9-10.
4. Samy RP, Gopalakrishnak one P. Therapeutic potential of plants as anti-microbials for drug discovery. Evidence-based complementary and alternative. Med. 2010;7(3):283-294.
5. Mahn A, Reyes A. An overview of health-promoting compounds of broccoli (*Brassica oleracea* var. *italica*) and the effect of processing. Food. sci. Tech. Int. 2012;18(6):503-514.
6. Wang X, Wu Q, Liu A, Anadón A, Rodríguez JL, Martínez-Larrañaga MR, Martínez MA. Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro. Drug. Metabol. Rev. 2017;49(4):395-437.
7. Pu S, Ren L, Liu Q, Kuang J, Shen J, Cheng S, He J. Loss of 5-lipoxygenase activity protects mice against paracetamol-induced liver toxicity. British j. Pharm. 2016;173(1):66-76.
8. Iwuozor KO. Quality assessment of selected Vitamin C tablets sold at Bridge Head Market, Onitsha. J. Chem. Biomolecular. Engineering. 2018;3(3):47-50.
9. Jäpelt RB, Jakobsen J. Vitamin D in plants: a review of occurrence, analysis, and biosynthesis. Frontiers in Plant Sci. 2013;4:136.
10. Charoenngam N, Shirvani A, Holick MF. Vitamin D for skeletal and non-skeletal health: What we should know. J. clin. Orthopaedics. Trauma. 2019;10(6):1082-1093.
11. Sounder L, Doss VA, Mohanasundaram S. Acetaminophen induced nephrotoxicity in experimental albino ratshistopathological study. Int. J. Res. Pharm. Sci. 2018;9(3):907-910.
12. Geetha TS, Geetha N. Phytochemical screening, quantitative analysis of primary and secondary metabolites of cymbopogancitratus (DC) stapf. Leaves from Kodaikanal hills, Tamilnadu, Int. J. Pharm. Tech. Res. 2014;6(2):521- 529.
13. Tietz NW.Text book of clinical chemistry.3rd ed.CA, Burtis ER, ashwood WB. Saunders. 1999:282-884:1429-1431.
14. Jacobson GA, Ives SJ, Narkowicz C, Jones G. Plasma glutathione peroxidase (GSH-Px) concentration is elevated in rheumatoid

arthritis: a case-control study. Cli. Rheumatol. 2012;31(11):1543–1547.
15. Armstrong DP, Browne R. The analysis of free radicals, lipid peroxides ,antioxidant enzymes and compounds related to oxidative in diagnostic medicine. Adv. Exp. Med. Biol. 1994;366:43–58.
16. Morgan G, Leech NA, Gloecner GW, Barrett KC. SPSS for introductory statistic:use and interpretation .2nd ed.Lawerenz Erlbum associatiates, publisers Mahwah,New Jersey Lonbon; 2010.
17. Abood AM, Awad HEA, Hassan SM. Potential role of vitamin D receptar agonist in acetaminophen-induced hepatic injury: role of caspase-1. Ain. Shams. Med. J. 2021;72(2):251-265.
18. Dusso AS, Tokumoto M. Defective renal maintenance of the vitamin D endocrine system impairs vitamin D renoprotection: a downward spiral in kidney disease. Kid. Int. 2011;79(7):715-729.
19. Najjar ZSR, Jwad SM. Assessment of 1.25(OH).2D3 levels and nephropathy stages in adult male patients with type -2 diabetes mellitus . J. Crit. Rev. 2020;7(13):1667-1674.
20. Raeeszadeh M, Zobeiri A, Khademi N, Mortazavi P. The study of the prominent nephroprotective effect of methanolic broccoli (*Brassica olaracea*, var. *Italica*) extract on oxidative-lead damage in mice kidney: biochemical parameters and pathological changes. Indian J. Exp. Biol. (IJEB). 2021;59(9):626-632.
21. Canayakin D, Bayir Y, Baygutalp NK, Karaoglan ES, Atmaca HT, Ozgeris FBK, Keles MS, Halici Z. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of *Nigella sativa*. Pharm. Biol. 2016;54(10):2082–2091.
22. Hamza RZ, Al-Harbi MS. Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. Asian .Pacific J. Tropical. Biomed. 2015;5(7):521-531.
23. Jasim SM, Jwad SM. Astaxanthin extract potentiates the functional performance of hepatic and renal tissues and can prevent or alleviate hepatotoxicity and nephrotoxicity induced by acetaminophen. Annal. The Roman. Soci. Cell. Biol. 2021:560-568.
24. Yayla M, Halici Z, Unal B, Bayir Y, Akpinar E, Gocer F. Protective effect of Et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. Eur. J. Pharm. 2014;726:87-95.
25. Abdul Hamid Z, Budin SB, Wen Jie N, Hamid A, Husain K, Mohamed J. Nephroprotective effects of Zingiber zerumbet Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. J. Zhejiang Universty. Sci. B. 2012;13(3):176-185.
26. Ghosh A, Sil PC. Anti-oxidative effect of a protein from Cajanus indicus L against acetaminophen-induced hepato-nephro toxicity. BMB Reports. 2007;40(6):1039-1049.
27. Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food . Chem.Tox. 2010;48(11):3246-3261.
28. Abdul-Zahra MT, Jwad SM. The effect of alcoholic effect of pomegranate peel on the level of erythropoietin and some of the blood properties in albino male rats treated with Erythromycin. Internat. J. Psychosoc. Rehabilit. 2020;24(04):6350-6361.
29. Köksal E, Gülçin İ. Antioxidant activity of cauliflower (*Brassica oleracea L.*). Turkish J. Agricul. Forestry. 2008;32(1):65-78.