Protective Role of Cranberry Extract Against Zovirax-Induced Spleen Dysfunction in Adult Female Wistar Rats

Imtithal Ali Mohammed [1], Eman S. Y. Al-Sarraj [2]
Zeena Dhubyan Mohammed Zaki [3]

Abstract

This research explored a possible protective function of antioxidants, such as cranberries, against zovirax-induced spleen dysfunction in adult female Wistar rats. A collection of 24(adult female Wistar rats) was haphazardly appointed to four equal sets, each including six animals. They received the following treatment for (0–22–44) days per day. The first group, known as group (C), was obtained in (tap water), and served as a control. The second set (A1) obtained orally 150 mg/kg B.W. of cranberry alone. The third group (A2) received zovirax (450mg/kg B.W.) during the trial to elicit spleen toxicity. In the fourth group (A3), zovirax (450mg/kg B.W.) was administered, plus cranberry (150mg/kg B.W.) was used to alleviate symptoms. Blood samples taken from the orbital sinus were obtained on days (0, 22, and 44) of the experiment, after a fast, to test the serum levels of albumin, total bilirubin (T.B.), globulin, and total serum protein (T.S.P.). After the experiment, splenic slices were taken out for histological analysis. The findings showed that the quantities of total blood protein, albumin, and total bilirubin significantly decreased in rats given zovirax (A2) at a significance level of ($P \leq 0.01$), accompanying histopathological investigation alterations regarding the histological composition of spleen tissue sections to treat all other sets. However, the protecting function of cranberries was elucidated in the set (A1), encompassing both the histopathological changes and correction of the spleen function mentioned above. Based on these facts, we have determined the results of this study confirmed that cranberries, as an antioxidant, can protect the spleen against zovirax-induced damage in adult females.

Key Words

Spleen; Cranberry Extract; Zovirax; Adult Female Wistar Rats.

Author Details

1 Department of Optical Techniques, Al-Noor University College, Mosul, Iraq
emtithal.ali@alnoor.edu.iq

2 Department of Pharmacy, Al-Noor University College, Mosul, Iraq
eman.sami@alnoor.edu.iq

3 Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq
zeena.dhubyan@uomosul.edu.iq
https://orcid.org/0000-0001-8901-6444

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Introduction

The spleen is a ubiquitous organ present in all vertebrates. It performs several crucial functions that maintain bodily health [1]. Its primary function is blood filtration, eliminating bacteria and shattered or damaged red blood cells. Its anatomy is akin to that of a sizable lymph node. [2], [3]. That organ plays a significant role in the immune system by generating white blood cells that combat infection and produce antibodies. Numerous illnesses, traumas, diseases, and abnormalities impact the spleen's function. In rat models, zovirax consumption results in necrosis, which then progresses to fibrosis and cirrhosis before becoming spleen cellular carcinoma. According to [4], zovirax is an antiviral and nucleoside analog used to treat varicella-zoster virus infections and herpes. Adults and children with severe infections brought on by the varicella-zoster virus (chickenpox virus), HSV-1, and HSV-2 are treated intravenously with zovirax [5].

Additionally, it is the recommended medication for managing herpes simplex encephalitis. Zovirax is often administered orally for treating adult and pediatric cases of chickenpox, managing the initial and recurring instances of mucocutaneous herpes in a specific patient, and treating cases of herpes zoster (shingles). Although systemic therapy is far more effective, mucocutaneous HSV infections are treated topically with zovirax. The body uses antioxidants as its first defense against free radicals and other oxidants. An antioxidant is a substance that can inhibit or delay the oxidation of other molecules. Avoiding oxidative stress in health and disease has garnered more attention recently. Oxidative stress denotes an initial occurrence in the pathology and physiology of chronic and non-communicable diseases [6]. Antioxidants effectively reduce the harmful effects of free radicals on oxidative damage to cells, which impacts the development and management of various disorders [7].

The World Health Organization reports that because plants have a wide range of bioactive chemicals that function as antioxidants in biological systems, 85% of nations, including those in Asia and Africa, use natural medicines derived from plants. An investigation into the effect of polyphenols on human health demonstrated that cranberry effectively suppressed oxidation lipids and prevented the creation of peroxidation products [8]. Eliminating free radicals, such as hydroxyl radicals(H.R.), superoxide radicals(S.R.), and singlet oxygen, by cranberry antioxidants is responsible for these benefits. This ultimately prevents the oxidation of biomolecules [9]. Among these plants are cranberries, which have long since valued for their therapeutic qualities. Native Americans Utilized (NAU) them to cure renal and bladder issues. Cranberries contain several bioactive components used widely in medicine because of their health benefits and ability to fight cancer. The components mentioned include fructose,

triterpenoids, vitamin C, flavonoids, anthocyanidins, catechins, and phenolic compounds [10]. According to research, cranberry flavonoids can remove harmful molecules called superoxide radicals, hydroxyl radicals, free radicals, and lipid peroxidation. Cranberry helps prevent damage to the mitochondria and loss of membrane integrity [11]. Powdered cranberry extract is added to food to strengthen the body's defenses against free radicals. Numerous biological characteristics of cranberries include their impact on microbial growth, biofilm formation, bacterial adhesion, immunomodulatory, and anti-inflammatory activity [12].

Moreover, cranberries were recognized to possess antioxidant qualities, as evidenced by their ability to protect red blood cells from hemolysis, maintain an antioxidant status in plasma, and exhibit cellular antioxidant activity [13]. Using this straightforward technique, it is possible to evaluate cranberry extract's antioxidant and protective properties against the oxidative stress that Zovirax causes in rats' spleen as part of an intriguing research program on the pharmaceutical importance of natural products, especially cranberry extract.

Aim of the Research

The objective of the current investigation was to assess the influence of cranberry on oxidative stress induced by zovirax in adult female Wistar rats. Explain the impact of Zovirax on specific biochemical indicators, including total protein, globulin, albumin, and total bilirubin. Additionally, discuss the effects of Zovirax on spleen tissue.

Materials and Methods

1. **Sampling:** In the animal house of the (College of Veterinary Medicine), adult female Wistar rats weighing between 190 and 250 grams were kept in cages with adequate lighting and ventilation. They were also provided with complimentary availability of water and regular rodent feed from the University of Mosul and Alnoor University College. They were given two weeks to get used to the experimental setup. For 44 days, twenty-four adult female rats were handled in the following ways each day.
2. **Experiment Design:** They were split up into four equal groups at random (6 rats in each group):
    1. The first set: designated as the control(C), was doused with tap water.
    2. The second set: (group A1) was administered orally with cranberry only (150mg/kg B.W.)[14].
    3. The third set (group A2): received zovirax (450mg/kg B.W.) to induce spleen toxicity during the experiment [15].

4. The fourth set (group A3): was treated with zovirax (450mg/kg B.W.) and oral cranberry (150mg/kg B.W.) to alleviate symptoms.

Fasting blood samples were obtained from anesthetized subjects using the retro-orbital sinus technique [16] at 22 and 44 days into the study period. The subjects were given an (i/m injection of ketamine 90mg/kg B.W. and Xylazine 40mg/kg B.W.) of serum was separated and refrigerated at -18 °C until analysis, after which samples were centrifuged for (15 minutes at 3000rpm). After that, the following parameters were assessed using kits (a BioSystems, Agappy, Switzerland product); these included albumin as reported by [17], and total serum protein (TSP) as calculated by [18]. State that the calculation of globulin involved subtracting (serum albumin) from (total blood protein), and total bilirubin(TB). After the experiment was over, the animals were killed, and portions of the spleen were taken out for histological analysis [19].

3. **Impact of Treatment on Biochemical Parameters:** Examinations Several ready tests were utilized to estimate the concentrations of biochemical parameters, including proteins, from multinational corporations such as the German Biocon Company, the French Biolabo Company, and the English Randox Company. Using the measuring techniques mentioned in Table 1, the amounts of various blood and biochemical parameters were estimated from the whole blood and serum (1). Manual techniques were employed to estimate the test of biochemical parameters.
4. **Histopathology studies:** Spleen tissue was fixed for a whole day in formal saline (10%). The tissue was then embedded in paraffin wax at (56ºC) after being dehydrated using (methyl, ethyl, and absolute ethyl alcohols). The wax tissue blocks were sectioned using a sliding microtome after hematoxylin and eosin staining, and they were then prepared for histological slide analysis using a light electric microscope (Olympus Cx21 with attached digital camera). Two separate investigators carried out morphometric analysis on ten sections of histology. Vacuolated hepatocyte counts (per square millimeter) and the percentage of lymphocyte-infiltrated surface (defined as the ratio of the infiltration area to the entire studied surface of the sample) were measured at ×100 and ×200 magnifications.
5. **Analytical statistics:** Results were analyzed using the complete randomized design(C.R.D.). Analyzed data was subjected to a thorough randomized design(C.R.D.). The groups were differentiated using the Duncan multiple range test, with findings evaluated at a concentration of ($P \leq 0.01$) and the statistical analysis conducted using the Microsoft Excel 365 software [20].

Results

1. Treatment's Impact on Biochemical Parameters:

Female rats were given zovirax (450 mg/kg B.W.) for 44 days. The statistics in tables 1, 2, and 3 demonstrate that their blood serum's albumin, total protein, and globulin levels were statistically significantly lower than those in the control(C) set. The concentration of total protein in treatment (A2) during the exposure period (22 and 44) days (4.99±0.17, 4.63±0.78 g/dl), respectively, decreases significantly at the level (P≤0.01) as indicated by (Table 1). When cranberries were added with the antibiotic and during the exposure period (22, 44) days, there was a modest rise in the level of total protein concentration in treatment (A3) to (5.60±0.32, 5.89±0.81 g/dl), respectively. Then, following treatment with cranberry antioxidants, a notable increase in the level of total protein concentration was seen during the same periods (22, 44) days in treatment (A1) to (6.17±0.97, 6.30±0.15g/dl), respectively. These outcomes closely resemble the control group's level.

Table 01- The protective effect of cranberry extract on total serum protein (TSP) concentration(g/dl) of adult female Wistar rats exposed to zovirax

Mean total serum protein (TSP) concentration (g/dl)				
Time	(C)	Groups (A1)	(A2)	(A3)
zero	6.18±0.38 A	6.26±0.45 A	6.33±0.48 A	6.26±0.36 A
22 days	6.34±0.42 A	6.30±0.15 A	4.99±0.17 BC	5.60±0.32 AB
44 days	6.41± 0.50 A	6.17±0.97 A	4.63±0.78 C	5.89±0.81 AB

Average ± Standard Error for three replicates. Different letters are positioned vertically before the numbers, signifying a significant difference at the probability level (P≤ 0.01) for each set of six animals. according to Duncan test.

When compared to the control(C) set, treatment (A2) during the exposure period (22, 44) days had a significant decrease in total albumin concentration(2.51±0.27, 2.25±0.056 g/dl) at the level (P≤0.01), according to the results of the measurement of total albumin concentration in the rats' blood serum, as shown in Table (2). while treatment (A3) zovirax with cranberry treated group showed a slight increase in total albumin concentration during the exposure period (22, 44) days (2.92±0.74, 3.14±0.83 g/dl) respectively. As its concentration was near that of the control group, treatment (A1) demonstrated a substantial rise in albumin concentration during the exposure period (22, and 44) days to the level of (3.67±0.33, 3.60±0.577 g/dl), respectively.

Table 02- The protective effect of cranberry extract on total serum albumin (g/dl) . of adult female Wistar rats exposed to zovirax

Mean total serum albumin concentration (g/dl)				
	Groups			
Time	**(C)**	**(A1)**	**(A2)**	**(A3)**
zero	3.53± 0.44 A	3.56±0.41 A	3.42±0.19 A	3.45±0.35 A
22 days	3.61±0.37 A	3.67±0.33 A	2.51±0.27 BC	2.92±0.74 ABC
44 days	3.57±0.46 A	3.60±0.577 A	2.25±0.056 C	3.14±0.83 AB

Average ± Standard Error for three replicates. Different letters are positioned vertically before the numbers, signifying a significant difference at the probability level (P≤ 0.01) for each set of six animals according to Duncan's test.

The efficacy of globulin content in rat serum is displayed in Table (3). The concentration of globulin in treatment (A2) decreased somewhat throughout the 22 and 44-day periods, coming in at (2.46±0.44 g/dl and 2.32±0.29 g/dl), respectively. Over the same period, the results were 2.64±0.21 g/dl for the control group and 2.61±0.15 g/dl for therapy (A1).

Table 03- The protective effect of Cranberry extract on total serum globulin (g/dl) of adult female Wistar rats exposed to zovirax

Mean total serum globulin (g/dl)				
	Groups			
Time	**(C)**	**(A1)**	**(A2)**	**(A3)**
zero	2.67±0.31 A	2.66±0.15 A	2.93±0.28 A	2.87±0.84 A
22 days	2.72±0.38 A	2.64±0.21 A	2.46±0.44 A	2.61±0.14 A
44 days	2.83±0.42 A	2.61 ± 0.15 A	2.32±0.29 A	2.80±0.14 A

Average ± Standard Error for three replicates. Different letters are positioned vertically before the numbers at the probability level (P≤ 0.01) for each set of six animals, according to Duncan's test.

2. Effect of Cranberry extract on total serum bilirubin

When compared to the control(C) set, the results in table (4) demonstrated a significant rise in bilirubin concentration at the probability level(P≤0.01) in the serum of rats treated with the antibiotic zovirax (A2) throughout periods (22, and 44). In treatment (A1), it dropped to 0.33±0.043 g/dl and 0.31±0.045 g/dl at the same exposure periods (22, 44), and the concentration resembled that of the control group. It climbed to 0.49±0.07 and 0.55±0.106 g/dl, respectively. The breakdown of red blood cells over the usual limit causes a rise in the percentage of total bilirubin in the blood, which is the cause of the high concentration of bilirubin in treatment (A2).

Table 04- The protective effect of cranberry extract on total serum bilirubin(TB) concentration(mg/dl) of adult female Wistar rats exposed to zovirax.

Mean total serum bilirubin (TB) concentration (mg/dl)				
	Groups			
Time	(C)	(A1)	(A2)	(A3)
zero	0.34±0.032 C	0.35±0.049 C	0.32±0.023 C	0.33±0.045 C
22 days	0.33±0.029 C	0.33±0.043 C	0.49±0.075 AB	0.40± 0.091 BC
44 days	0.34±0.032 B	0.31±0.045 C	0.55±0.106 A	0.42±0.056 BC

Average ± Standard Error for three replicates. Different letters are positioned vertically before the numbers, signifying a significant difference at the probability level (P≤ 0.01) for each set of six animals. according to Duncan test.

3. The histopathological results of the spleen tissue in various rat groups show:

The spleen's normal anatomy is depicted in Figure 1 of the histological evaluation. Sections of adult female Wistar rats in the control group showed visible white pulp cells and red pulp cells with a central artery.

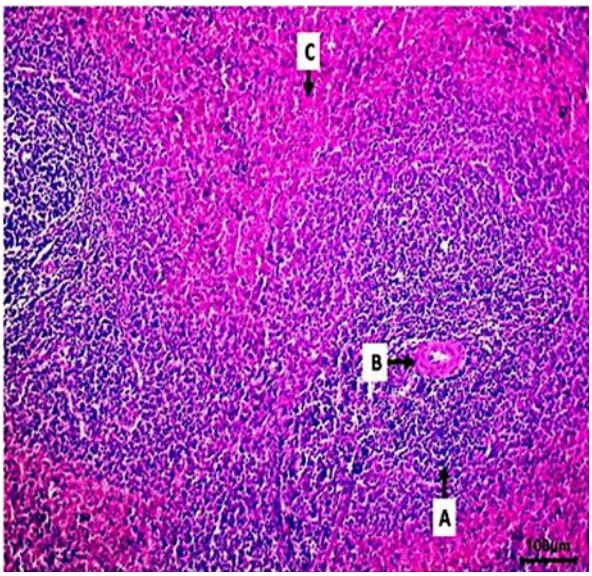

Figure 01- Photomicrograph of rat's spleen of the control group showing normal architecture of white pulp (A) with central artery (B) and red pulp (C) .H & E stain, 100X [21].

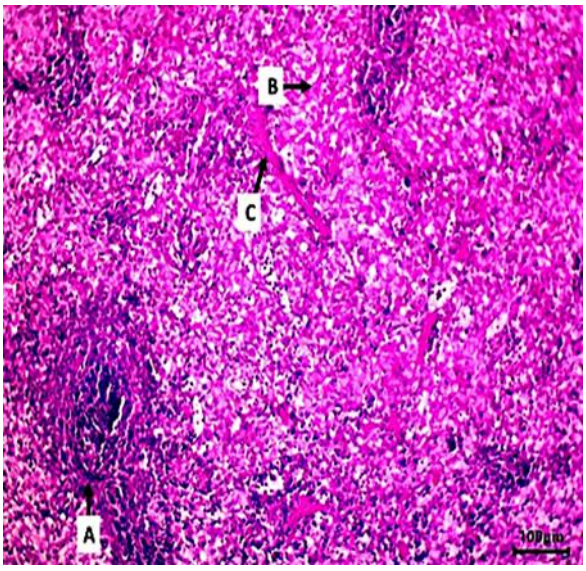

Figure 02- A photomicrograph of a rat's spleen from the zovirax-treated group demonstrates cell loss and necrosis in the red and white pulps (A and B), together with fibrinoid necrosis in the ellipsoidal sheath shown in (C).Stain H&E, 100X [22].

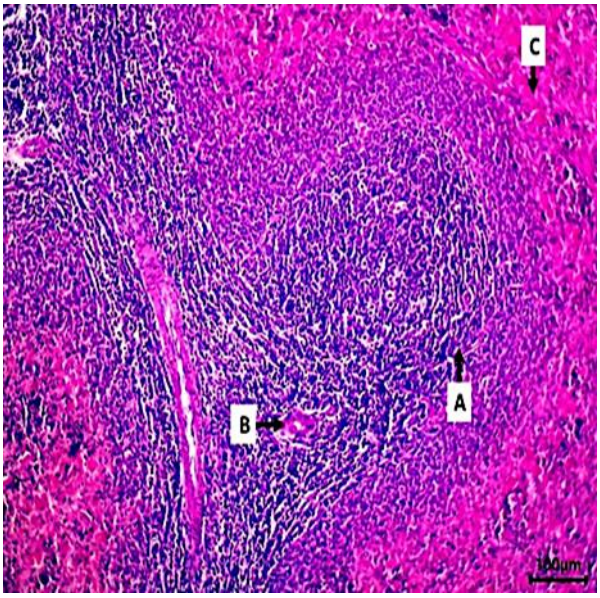

Figure 03- Photomicrograph of rat's spleen of the Zovirax with Cranberry treated group showing normal architecture of white pulp (A) with central artery (B) and red pulp (C) .H & E stain, 100X [23].

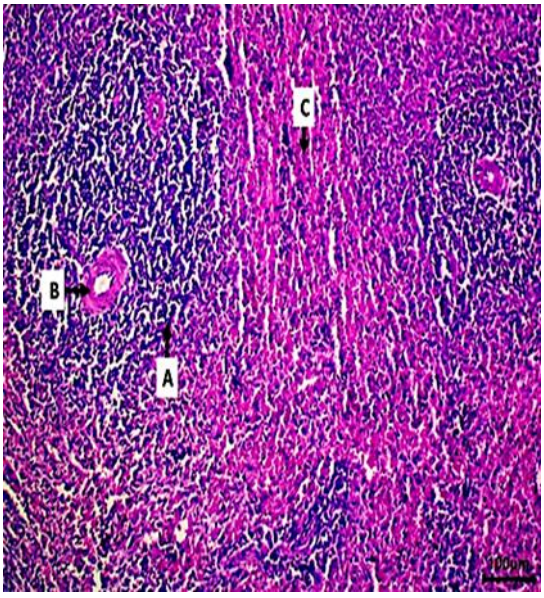

Figure 04- A photomicrograph of the rat spleen from the Cranberry group demonstrates the typical architecture of the red pulp (C) and white pulp (A) with the central artery (B).100X H&E stain [24].

Discussion

The current manuscript investigates the anti-fibrotic properties of cranberries by evaluating different pharmacological, biochemical, and pathomorphological factors in rats with spleen fibrosis produced by Zovirax. This model is extensively accepted and used to study hepatic fibrosis and screen hepatoprotective agents [25]. Solutions containing berry polyphenols have been demonstrated to have cytoprotective effects on the myocardium [26]. Interestingly, the current study's findings support cranberry's protective effects against spleen damage. Cranberry extracts reduce fibrosis and inflammation to lessen Zavriax-induced spleen damage. Compared to the control(C) set, the current study's results indicate a significant decrease in (blood serum protein) levels during the 0, 22, and 44-day treatment periods with the antibiotic zovirax. This decrease may result from the antibiotic interfering with the body's processes of protein synthesis and metabolism, or it could result from an osmotic imbalance brought on by the release of high concentrations of lipid peroxide [27].

The protein degradation that occurs due to the antibiotic's impact on the structural metabolism of proteins in the liver and spleen could be the cause. Because cranberries contain flavonoids, which are very good at reducing spleen damage, using them resulted in a rise in protein levels. These flavonoids prevent or suppress free radicals, which also maintain proteins

[28]. In an investigation of the cranberries' active ingredients and a test of the spleen's blood proteins.

This is a crucial sign for bolstering the defenses against illness and determining the organism's state of health. The findings of this investigation support those of numerous other studies, which show that cranberry extract's potency stems from its high phenolic component content [29]. Furthermore, anthocyanins are critical in lowering the pathogenic activity of root growth. Free oxygen is released into the bloodstream when the body is subjected to substances that damage its tissues or normal metabolic processes occur, according to [30]. Phenolic phytochemicals include phenolic acids (benzoic, hydroxycinnamic, and ellagic acids). Moreover, cranberries have abundant flavonoids (anthocyanins, flavonols, and flavan-3-ols). Cranberries and their products have been found to possess some of the most significant antioxidant capacities among fruits, according to studies that have employed several antioxidant activity measurements [31] [32].

The albumin, globulin, and total protein concentrations significantly increased in the rats treated with cranberries. Therefore, cranberries can be a characteristic to several factors, including the ability of antioxidants such as flavonoids, phenolic compounds, and vitamin C to promote the process of protein synthesis in many organs, especially the liver and spleen, or to the role of cranberries in this regard. That is something that the cranberry contains and is significant. Because they all aim to reduce the use of albumin as an antioxidant, stop the oxidation of proteins and amino acids, and eliminate free radicals, oxidative stress is reduced [33].

The two main components of the spleen are the red and white pulps [34]. The white pulp mainly comprises white blood cells that are important at the start of the adaptive immune response. It is composed of lymphatic tissue that surrounds a central arteriole. B-cells are found in the germinal core, the deepest part of the white [35]. T-cells are present in the periarteriolar lymphoid sheath, which envelops the marginal zone. Red pulp encircles the white pulp seen throughout the spleen. The structure's distinct red appearance under a microscope is caused by a significant number of venous sinuses and splenic cords, or Cords of Billroth.

The reticulin and fibrils produced by the splenic cords provide the organ structure. Additionally, a supply of monocytes that promote wound healing is present in the cords. The splenic cords lead to the splenic sinuses, home to macrophages. They react to antigens and remove aged or aberrant erythrocytes from the bloodstream. The dorsal mesogastrium, referred to as the larger omentum, is home to a mass of mesenchymal cells from which the spleen is

derived. [36]. To compare with the control group, the treatment (A2) showed changes in fatty tissue, vacuolar degeneration, and cell death in the ellipsoidal sheath (Figure) of the red pulp (B) and white pulp (A). In contrast, these tissue characteristics vanished following (A1) cranberry treatment. The rationale is that, as shown in Figure (4), cranberries' ability to shield tissue from harmful substances to which it is exposed because of consuming antibiotics helps to shield the body and its tissues from the harmful effects of an antibiotic. Cranberry extract has been shown to maintain the histological integrity of damaged spleen tissue with parenchyma necrosis, tubular extension, and hyperemic conditions [37].

Cranberry extract was used to improve the structure and reduce the rigidity of the histopathological change in treated rats. It also partially restored the studied parameters to standard values (Figure 4). Flavonoids were discovered during a phytochemical preliminary investigation of cranberry extract. Bioflavonoids, also known as flavonoids, are chemical molecules that can impact many cellular processes and regulate the SOD through the enzymatic activity of superoxide dismutase and catalase. In many research investigations. Cranberries have been linked to antibacterial, antiviral properties, antiangiogenic, anticarcinogenic, antimutagenic, antioxidant, and anti-inflammatory effects [38].

Conclusions

We concluded that the results of this study supported the notion that cranberries, as an antioxidant, can shield adult female Wistar rats' spleens against harm caused by zovirax. Zovirax impacts biochemical measurements such as total protein, globulin, albumin, and total bilirubin. Cranberry extract's spleen-protective properties against oxidative stress brought on by zovirax in female rats. As a component of a fascinating study on the value of natural goods in medicine, particularly cranberry extract. The antibiotic's effects on the liver's and spleen's structural metabolism of proteins may cause protein degradation. Using cranberries increases protein levels because they contain flavonoids, which are excellent at reducing spleen damage. These flavonoids preserve proteins and completely prevent or reduce free radicals. The female rats given cranberry treatment had far higher concentrations of albumin, globulin, and total protein. Cranberries help protect the body and its tissues from adverse effects by shielding tissue from toxins to which it is exposed due to eating antibiotics.

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Conflict of Interest: We confirm that this document is free from any conflict of interest.

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