



Immunohistochemical study of Whey Protein in the testis of Albino Rats

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Abstract

The present study was conducted to determine the immunohistochemical changes of whey protein on the male albino rats. Different concentrations were prepared from whey protein were imported from Sports Center for Nutritional Supplements in Baghdad, which mixed with distilled water and used directly for orally administration. Adult 36 male albino rats were randomly divided into three groups (12 as control and 12 treated with (0.8 g and 12 treated with 1.6 g whey protein, respectively). After 60 days, animals were sacrificed and testis and epididymis were isolated and fixated for immunohistochemical study. The immunohistochemical results in the testis showed increased in the percentage of T lymphocytes which marked by CD3 marker especially in the highest concentration (1.6) g. Also, there was positive staining of B lymphocytes which marked by CD20 marker in the seminiferous tubules and interstitial tissue. In addition, there was weak staining of plasma cells which marked by CD138 marker in the interstitial tissue only. Whereas the vimentin test showed no immunohistochemical changes were found in the seminiferous tubules and interstitial tissue comparing with control group.

Keywords: Immunohistochemical, CD3, CD20, CD138 and vimentin.

DOI Number: 10.14704/nq.2022.20.6.NQ22721

NeuroQuantology 2022; 20(6):7179-7186

Introduction

The biological components of whey, including lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycopeptide and immunoglobulin, exhibit a range of immune-enhancing properties. In addition, whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent. The primary mechanism by which whey is believed to exert its effect is the intracellular conversion of cysteine to glutathione, a powerful intracellular antioxidant (Marshall, 2004). Which could play a further role in its physiological effects. Whey is a protein complex derived from milk, making approximately 20%, and casein is the other protein that forms approximately 80% of the total protein content. The most common forms of whey protein used in high protein bars, beverages, and supplements are the concentrate (WPC) or the isolate (WPI). Whey protein is a complete, high protein with a rich amino acid profile. It contains the full spectrum of amino acids including essential amino acids



(EAAs) and branched-chain amino acids (BCAAs) which are important in tissue growth and repair. Leucine is a key BCAA in protein synthesis and has recently been identified as playing a critical role in muscle building, and glucose metabolism. Whey protein also has important benefits like it reduces the symptoms of chronic infections as it increases the immunity (Gangurde, 2011). Consumption of whey protein seems to play an anti-obesity and muscle-protective role during dieting by increasing thermogenesis and maintaining lean mass. In addition, whey protein has been shown to improve glucose levels and insulin response, promote a reduction in blood pressure and atherosclerosis, and improve lipid profile (Pal, 2013). Thus, they are widely used in the food industry. Accumulation of intracellular reactive oxygen species (ROS) during extended periods of oxidative stress is associated with the development of many chronic diseases (He F, 2012). Most of the youngsters are passionate gymnasts and bodybuilders who play sports and tear their muscles on a daily basis. Mobility, rotator cuff muscle weakness, and training load are important modifiable factors associated with body injuries (Tooth, 2020). Young people use protein supplements daily. Protein is what helps the body rebuild its muscles together and thus plays a major role in strengthening the human body. Whey protein contains the highest percentage of protein source and BCAAs (Carrilho, 2013). The study aimed to explore the immunohistochemical effects of different concentrations from Whey protein on the testis of male albino rats.

Materials and Method

Whey protein collection and preparation

The whey protein utilized in Iraq are always imported, entire products that are made abroad. Whey protein was collected from private sport centers in Baghdad. In this study, different doses of whey protein were used (0.8 and 1.6) g. These doses were prepared in accordance with the human dose (2-3 g/kg of body weight) (Campbell BI, 2018).

Animal and Experimental protocols

The animals were obtained from the laboratory animal center of AL- Qadisiya University. The rats were housed in normal laboratory conditions and had free access to food and drinking water. 36 albino rats were randomly divided into 3 groups. Each group contains (12) rats in a separate cages. The doses (0.8) g was given to group 1, and (1.6) g was given to group 2, and compared to control group. The treatment continued for sixty days.

Immunohistochemical study

After sixty days, animals were sacrificed and testis were isolated for immunohistochemical studies. Testis were fixated with a 10% concentration of

formalin solution for 24 hours and immunohistochemical sections were prepared at 4 μ m to detect the T lymphocyte, B lymphocyte, plasma cells and vimentin by using the methods of an avidin-biotin complex with the monoclonal antibody CD3, CD20, CD138, and Vimentin (Patho sito Leica company) (Suvarna, 2013). The sections of testis were examined by Olympus light microscope. T , B lymphocytes, plasma cells and vimentin were counted per 10 high power fields (400x) and then mean percentage for each group were calculated. The score of counting followed:

++++: more than 50% (very intense)
+++ : 25-50% (intense)



- ++: 10-25% (Moderate)
- +: 0-10% (weak)
- : no cell (Negative) (Xie Y, 2008)

Results:

The sections of testis were examined in a light microscope and showed that the infiltrates in the testis contained especially CD3 T lymphocytes were weakly positive, and included about (1–2%) cells in seminiferous tubules and in interstitial tissue

of the control group (Figure1A). While, the testis of animals treated with dose (0.8 g) of whey protein showed weak staining score included (1–2%) of T lymphocytes in seminiferous tubules and (2-3%) in interstitial tissue (figure1B). The testis of treated animals with (1.6 g) of whey protein showed positive staining with score (1–3 %) of T lymphocytes in seminiferous tubules and (3–4%) in interstitial tissue (figure1C).

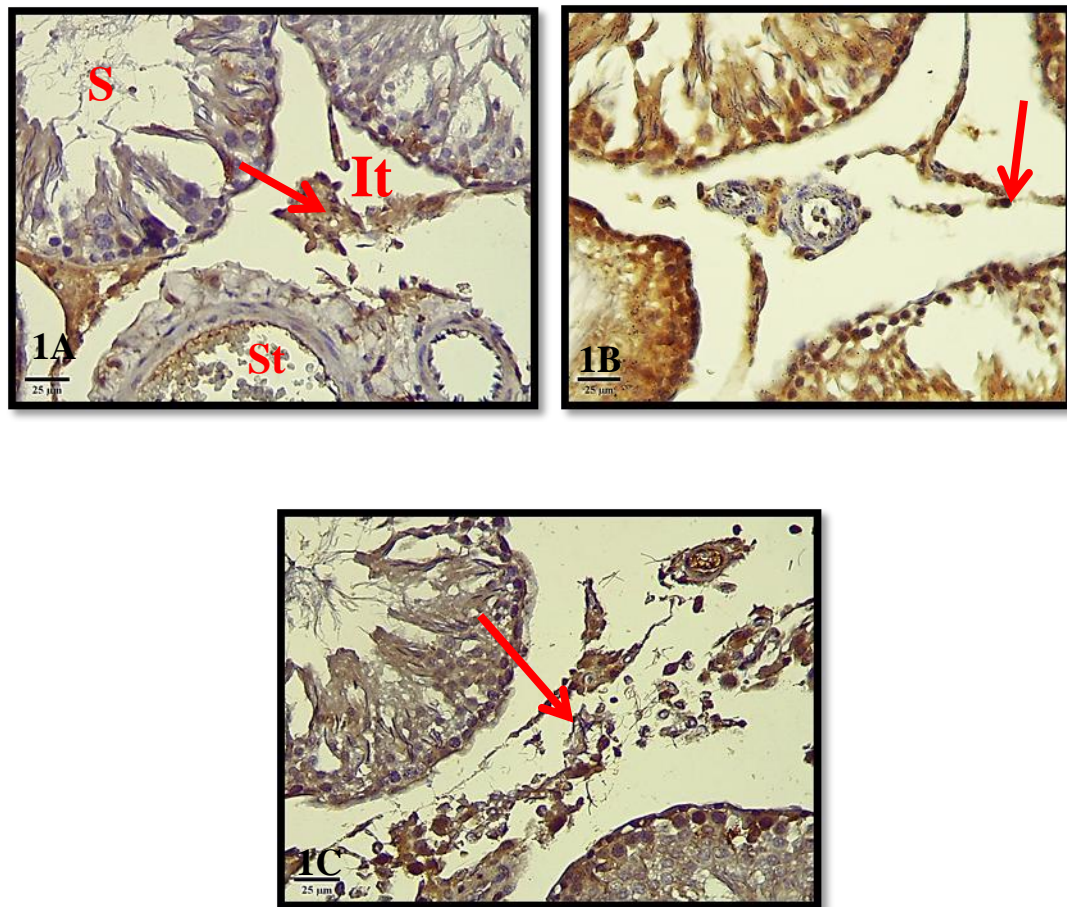


Figure1: Sections of testis showing CD3 expression on A: Sperm (S), Interstitial tissue (It), Seminiferous tubules (St). Contain weakly positive (1-2%) CD3 T lymphocyte in control group. B: Sections of testis of rat treated with (0.8 g) dose from whey protein showing (1-2%) CD3 T lymphocyte in interstitial tissue. C: Sections of testis of rat treated with (1.6 g) dose from whey protein showing (1– 3 %) of CD3 T lymphocytes in seminiferous tubules and (3 – 4%) in interstitial tissue.

Few scattered of CD20 B lymphocyte (0 – 1%) in the seminiferous tubules with little positive (1–3%) in the interstitial tissue in the testis of control group (figure2A). While, the testis of the animal treated with dose (0.8 g) of whey protein showed positive staining

with score (1–2%) in seminiferous tubules and (1– 3%) in interstitial space (figure2B). Additionally, in the testis of treated group with (1.6 g) of protein showed (1– 3%) in seminiferous tubules and (3– 4%) in interstitial space (figure2C).

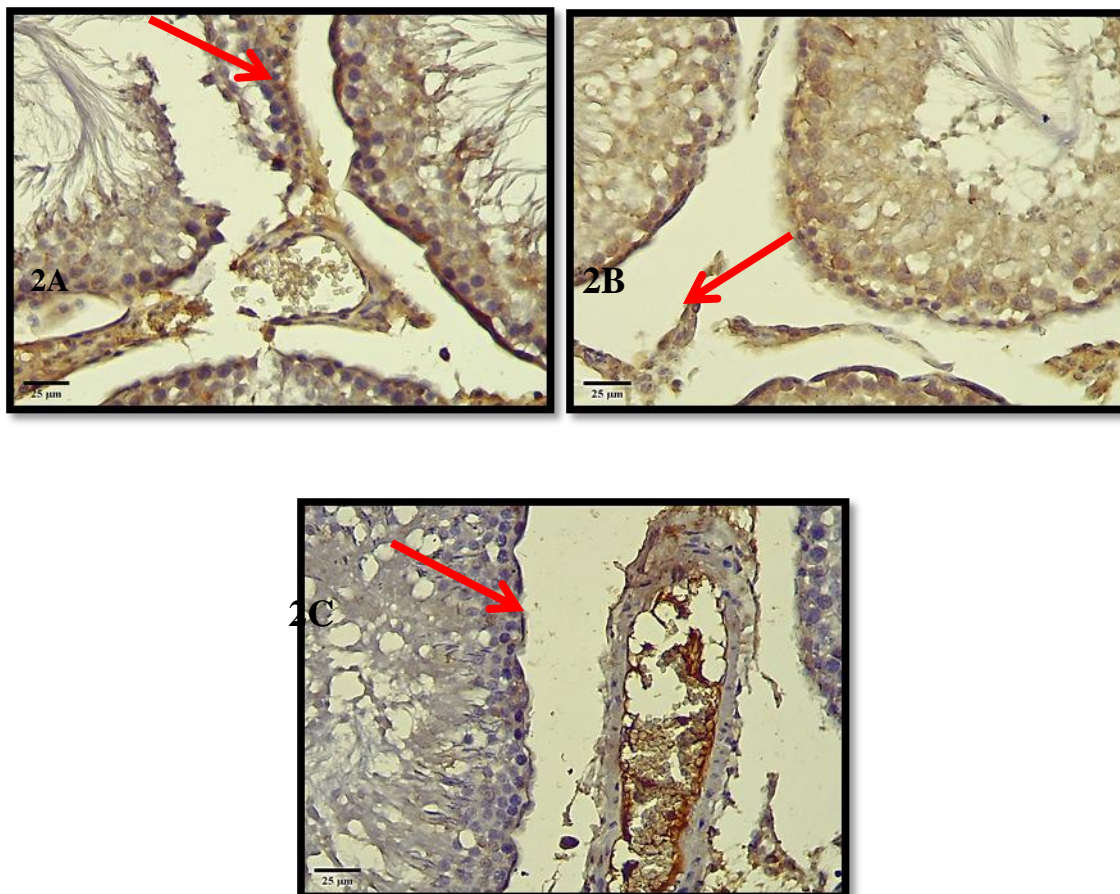


Figure2: Sections of testis showing CD20 expression on A: Contain weakly positive (1-2%) CD20 B lymphocyte in interstitial tissue (arrow) in control group. B: Sections of testis of rat treated with (0.8 g) dose from whey protein showing (1-2%) CD20 B lymphocyte in interstitial tissue (arrow). C: Sections of testis of rat treated with (1.6 g) dose from whey protein showing (3–4%) of CD20 B lymphocytes in interstitial tissue (arrow).

CD138 antibody exhibited weak staining (1 – 2%) in control group. (Figure3A). And weak in treated animal group 1 (0 – 2%) in the interstitial tissue (figure3B). The males in group 2 treated with whey protein showed weak staining for CD138 antibody (1 – 2%) (Figure 3C).

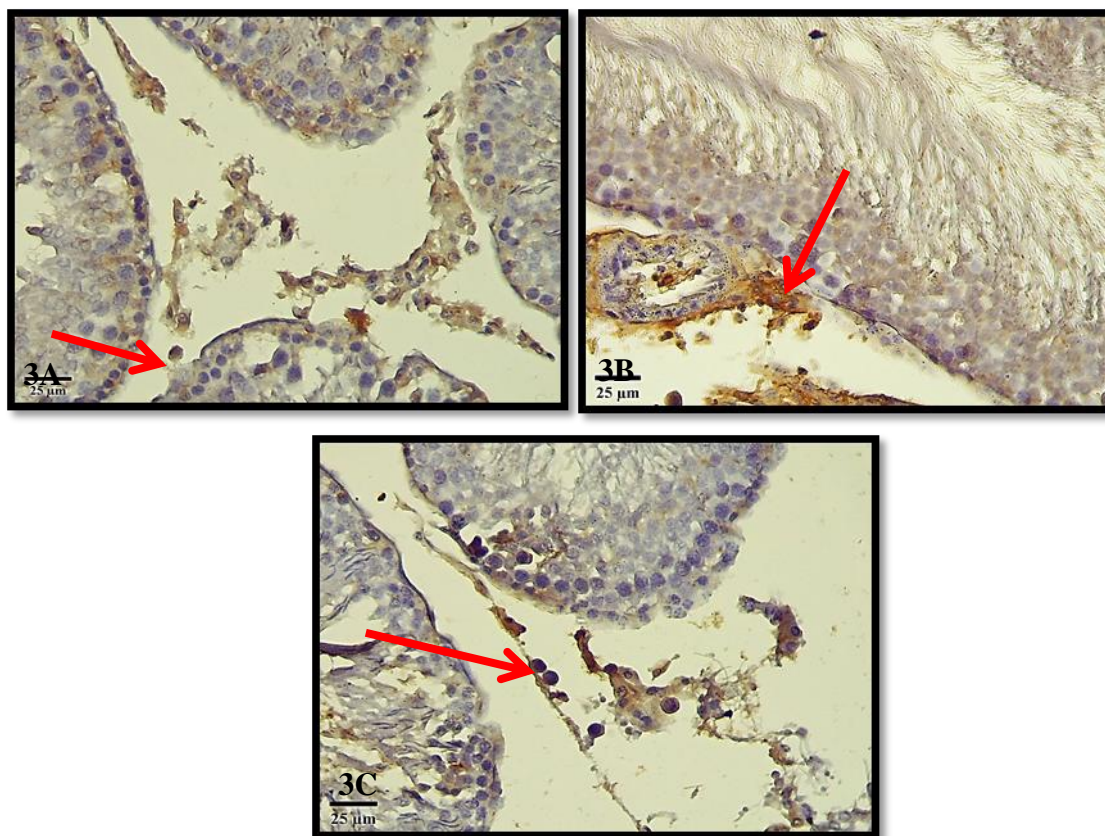
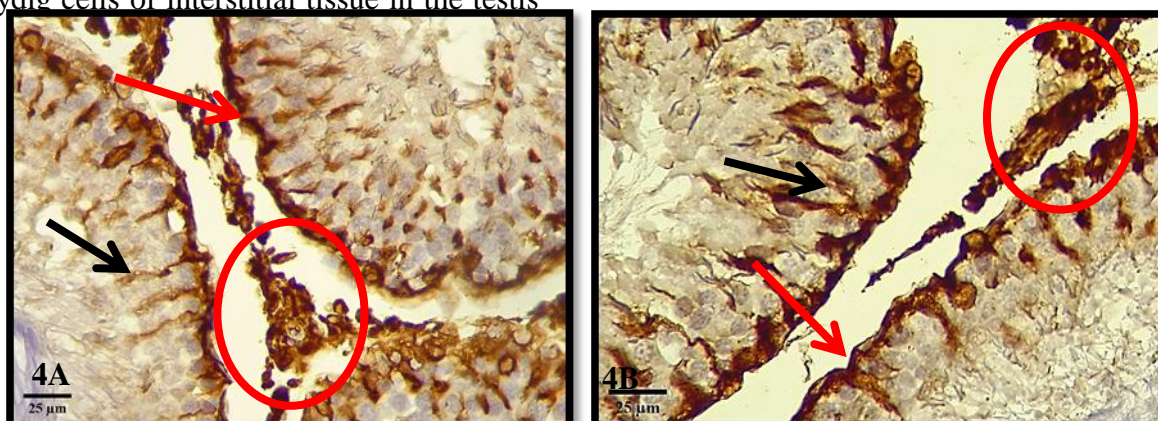


Figure3: Sections of testis showing CD138 expression on A: Contain weakly positive (0-1%) CD138 plasma cell in interstitial tissue (arrow) in control group. B: Sections of testis of rat treated with (0.8 g) dose from whey protein showing (1-2%) CD138 plasma cells in interstitial tissue (arrow). C: Sections of testis of rat treated with (1.6 g) dose from whey protein showing (1-2%) of CD138 plasma cells in interstitial tissue (arrow).

Normal positive reaction with the vimentin antibodies was found in basal layer of seminiferous tubules, Sertoli cells and in Leydig cells of interstitial tissue in the testis

of control group (figure4A). The treated group 1 and 2 show no change and look like control group (figure 4B and 4C).



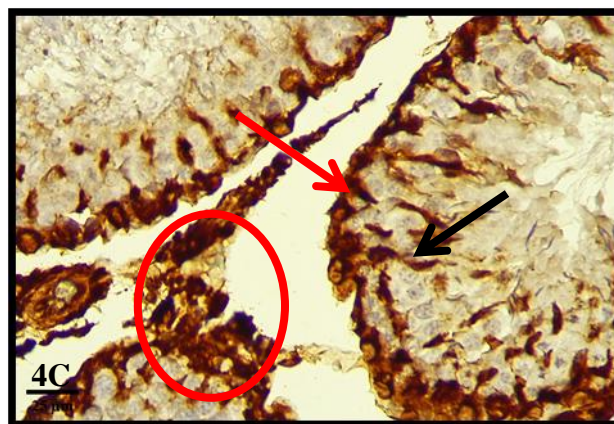


Figure4: Sections of testis showing vimentin expression on A: Contain normal positive in the basal layer of seminiferous tubules (red arrow), Sertoli cells (black arrow) and in Leydig cells (circle). B: sections of testis in rats treated with (0.8 g) dose from whey protein showing similar to control group. C: Sections of testis in rats treated with (1.6 g) dose from whey protein showing similar to control group.

Discussion:

Increase CD3 T lymphocytes proliferation in testis of male albino rat would be observed in the presence of different concentration from whey protein, perhaps due to tissue damage that leads to stimulating cytokine secretion, which leads to the accumulation of lymphocytes at the site of inflammation as an immune response. There is another explanation, L-arginine amino acids modestly increased interleukin IL-2 production and may increase T lymphocyte proliferation in mice by increasing specific receptor expression and the utilization of interleukin-2 to enhance cellular immune response (Ochoa, 2001). (Ivkovic, 2004) suggested that the dietary supplements was associated with an increased CD3 T lymphocyte count, due to

the effects of oral supplementation with the natural zeolite on the immune system of patients. While the testis showed increased CD20 B lymphocytes proliferation in treated animals, may be the protein caused increased B lymphocytes due to stimulate immune response or it is used the protein as energy source for synthesis, the result is agreement with (Venkatraman, 2002) when the athletes used glutamine that caused increased numbers of B lymphocytes, because the lymphocytes oxidized the protein as fuel for synthesis of DNA. (Van Gorkom , 2018) discussed provide ascorbic acid has multiple effects on the development, proliferation and function of lymphocytes in mice, There are important indications that ascorbic acid acts as an epigenetic regulator/cofactor. Plausibly,



ascorbic acid's epigenetic functions are mostly seen in cells that undergo change. In situations of cells under stress, the antioxidant properties of ascorbic acid are probably more important. Here, ascorbic acid could enhance immune reconstitution after treatments that give long immunosuppression as most studies indicate a positive effect of ascorbic acid supplementation on lymphocyte development. CD138 plasma cells expression showed increased when increased whey protein concentration in treated animals, this can be explained by the fact that the B cells recognized the influencing factor and treated it as an antigen. After encountering the antigen, B cells are activated and differentiate into plasma cells. The number of plasma cells increases in mice as the concentration of the influencing factor increases (Wang, 2021). (Bendich, 1986) suggested that dietary vitamin E significantly affected lymphocytes and plasma cells response to stimulated and proliferation in mice. Normal positive reaction with the vimentin antibodies was found in basal layer of seminiferous tubules, sertoli cells and leydig cells of interstitial tissue in the testis of treated groups similar to control group.

Conclusion

The whey protein used in this study cause severe effects on testis. So, further studies are necessary to investigate the physical and chemical compositions of whey protein and their effects on other organs.

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