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Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 33, Number 2, Pages 109-122 (2024)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1424347

*Research Article; Received: February 22, 2024; Accepted: May 09, 2024*

# INVESTIGATION OF LECTIN BINDING ON RABBIT SPLEEN CELL MEMBRANE INFECTED WITH *Proteus vulgaris*

MARYAM DIANI[1], HAKAN ESKİZENGİN[1], MOHAMMAD NIMA ARIAFAR[1], NURSEL GÜL[1]

[1]Department of Biology, Faculty of Science, Ankara University, Ankara, TÜRKİYE

**ABSTRACT** This study investigated the effects of *Proteus vulgaris* OX19 infection on the carbohydrate composition of spleen cell membranes in New Zealand adult male rabbits. Rabbits were injected with increasing doses of *P. vulgaris* OX19 (0.5 ml, 1 ml, 2 ml, 4 ml, 5 ml) at five-day intervals over the course of one month. Following the treatment period, spleen tissues were collected from both the control and infected groups. Tissue sections were stained using the Avidin-Biotin-Peroxidase method with five different lectins: *Concavalia ensiformis* (Con A), *Arachis hypogaea* agglutinin (PNA), *Bauhinia purpurea* agglutinin (BPA), *Griffonia simplicifolia* I (GS-I), and *Ulex europaeus* agglutinin I (UEA-I). The stained sections were examined by light microscopy to evaluate lectin binding. Among the lectins used, Con A showed strong binding (+++) to spleen cell membranes of the *Proteus*-infected group, while moderate binding (++) was observed in the control group. UEA-I exhibited weak binding in the control group but demonstrated moderate binding in the *Proteus*-infected group. In contrast, PNA, BPA, and GS-I exhibited strong binding (+++) to spleen cell membranes in the control group and moderate binding (++) in the infected group. These findings suggest that *P. vulgaris* OX19 infection induces alterations in the carbohydrate moieties of glycoproteins and glycolipids in the spleen cell membranes of infected rabbits. It is hypothesized that *P. vulgaris* modifies the terminal carbohydrates of glycoproteins and/or glycolipids in spleen cell membranes, contributing to the observed changes in lectin binding patterns.

**Keywords** *Proteus vulgaris*, rabbit, spleen, lectins, histochemical staining, light microscopy

## 1. INTRODUCTION

*Proteus* species are part of the Enterobacteriaceae family of gram-negative bacilli. *Proteus* organisms are implicated as serious causes of infections in humans, along with *Escherichia*, *Klebsiella*, *Enterobacter*, and *Serratia* species. Enterobacter strains are among the leading causes of nosocomial infections. They cause a wide variety of infections in humans, especially in the lungs, urinary systems and surgical wounds [1, 2]. *Proteus* is a normal flora element in the human intestine and is therefore frequently found in sewage. Urinary tract infections caused by *Proteus* bacteria are long-term infections and can cause kidney stone formation [3]. Frequent urinary tract infections in humans are probably due to the ability of *Proteus* to rapidly break down urea [4]. *Proteus* genus bacteria can be isolated in meningitis, sepsis and organ abscesses. It

diani.m11@gmail.com- 0009-0009-1928-0587 https://ror.org/01wntqw50
eskizengin@ankara.edu.tr- 0000-0003-1709-7497 https://ror.org/01wntqw50
m.nimabadali@gmail.com- 0000-0003-3122-9695 https://ror.org/01wntqw50
ngul@science.ankara.edu.tr- Corresponding author; 0000-0003-2978-4163 https://ror.org/01wntqw50

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usually causes co-infections with other bacteria in hospitals [5]. Also, *Proteus* bacteria can cause umbilical cord infections in newborns and sepsis and meningitis, which can occur as epidemics resulting from these infections [6-8].

Lectins are proteins/glycoprotein molecules in the extracellular matrix and cell membranes that bind complex sugar chains with high affinity but do not have an enzymatic activity against the bound sugar residues [9-13]. It has been determined that lectins, obtained from plants and able to bind to sugar residues in the surface glycoproteins and organelles of cells, stimulate the immune system cells and increase their cell number and activity [14-16]. Many metabolic events and structural features occurring in living things are determined by commercial lectins obtained from various sources [17]. Lectin histochemistry can therefore be used to visualize different cellular glycosylation patterns which is helpful elucidating the structure, physiology and pathology of tissues [18]. For example, species identification and detection of diseased cells are made using labeled lectin [19].

As a laboratory animal, the rabbit has an immune system that is immunologically similar to humans. The spleen, as an immune system organ, is where macrophages and B and T lymphocytes are located and where both cellular and humoral immune responses occur in infections [20, 21].

## 2. MATERIALS AND METHODS

### 2.1 Bacterial culture

Bacterial culture of *Proteus vulgaris* OX19 (Pasteur Institute No:54160) was obtained from Refik Saydam Hıfzıssıhha Center. Bacteria were maintained on Nutrient Broth including %1 glucose and incubated at 37°C.

### a. Experimental Animals

In the study, the care and animal experiments of New Zealand adult male rabbits were carried out at the Refik Saydam Hygiene Directorate Experimental Animal Production Center. Each animal was growth in separate cages under laboratory conditions with an appropriate photoperiod (14:10 hours of light/darkness) and a temperature of 20±2ºC [22]. In the experiments, 5 of a total of 10 rabbits weighing 2.5±0.4 kg were used as the control group and 5 as the *Proteus* treated group.

### b. Bacteria Injection

At the end of the logarithmic phase, the bacteria were centrifuged and diluted with physiological saline solution (0.9% NaCl) at Mc Ferland density, that is, 2.109 bacteria/ml. Bacteria were injected into rabbits at five-day intervals, with the first dose subcutaneously (0.5 ml) and other doses (0.5 ml, 1 ml, 2 ml, 4 ml, 4 ml) intravenously. Control group animals were injected with the same amount of physiological saline solution [23].

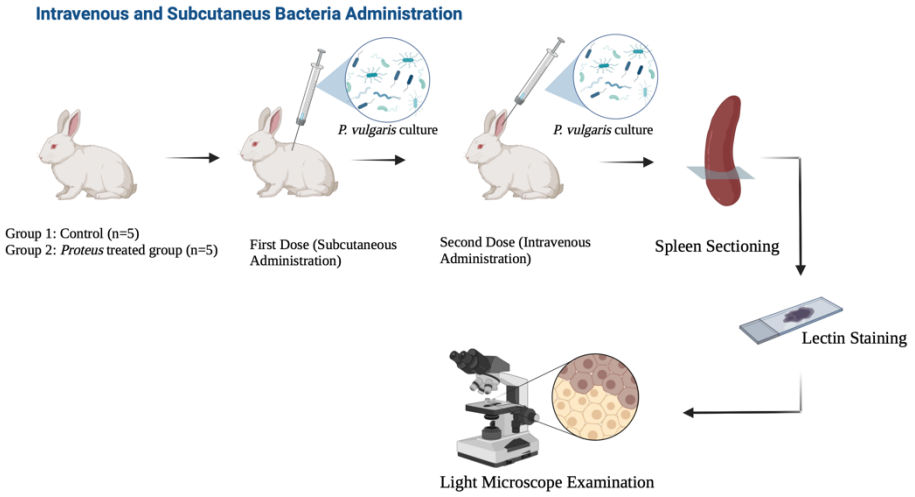


FIGURE 1. Schematic representation of the implementation stages of the experiment (Created with BioRender.com)

### c. Lectin Staining Method

Spleen samples taken from control group and *Proteus*-treated rabbits were fixed in 10% formaldehyde solution for 24 hours. Then, the samples were washed with distilled water and dehydrated by passing through the Alcohol series (70%, 80%, 90%, 100%, 100% alcohol) and placed in Paraffin embedding medium. 4µm thick sections were taken from the blocks in the paraffin embedding medium with a microtome and placed on a polylysine slide. The sections taken on a polylysine slide were kept in a 70°C oven for 30 minutes to melt the paraffin. Paraffin residues in the sections were removed by keeping them first in pure xylene and then in 96% alcohol for 30 minutes [24, 25]. In the experiments, Phosphate Buffer Saline (PBS) (pH:7.4) was used both in the preparation of the solutions and in washing the sections during the staining process [26]. The sections were first kept in $H_2O_2$ for 10 minutes to block endogenous peroxidases, and then kept in lectin solutions prepared at certain concentrations for an hour (Table 1).

TABLE 1. List and concentrations of lectins used in the experiment.

| **Lectins** | **Specific carbohydrates they bind to Lectins** | **Lectin (µg/ml)** |
|---|---|---|
| *Canovalia ensiformis* (Con-A) | α-D- Mannose > α-D-Glucose | 2.5 |
| *Arachis hypogaea* (PNA) | β-D galactose, β-D N-Acetyl galactosamine | 5.0 |
| *Ulex europaeus* (UEA-1) | α-L-Fucose | 5.0 |
| *Griffonia simplicifolia* (GS-I) | α- D- Galactose | 5.0 |
| *Bauhinia purpurea* (BPA) | β-D- Galactose, β-D N-Acetyl galactosamine | 5.0 |

After this process, the sections were washed 3 times with PBC. The sections were incubated with Avidin-Biotin Peroxidase enzyme complex for 45 minutes and then washed three times with PBS. Then, the sections were incubated in 0.6 mg/ml Diaminobenzidine (DAB) prepared with 3µl $H_2O_2$ for 5 minutes [27]. Finally, the sections were stained with Harris Hematoxylin dye (for counterstaining) for 1 minute and were examined under a light microscope (Nikon Eclips 50i).

## 3. RESULTS

In this study, the structural changes caused by *Proteus vulgaris* OX19 strain in the carbohydrates of glycoproteins and glycolipids in rabbit spleen cell membranes were examined under light microscope with histochemical method. *P. vulgaris* OX19 strain, known as an infectious agent, was injected into rabbits. One month after the bacterial injection, spleen samples taken from the control group and *Proteus* group rabbits were embedded in paraffin and sectioned. Paraffin sections were stained with five types of lectins according to the Avidin-Biotin Peroxidase enzyme complex method. The staining intensity of cell membranes with lectins was evaluated under light microscopy. As a result of the observations, Con A, which specifically binds to mannose and glucose, was bound to the spleen cell membranes of the control group rabbits with moderate strong (++) (Figure 1), and unlike the control group, it was strongly (+++) bound to the spleen cell membranes of the rabbits injected with *P. vulgaris* OX19 bacteria (Figure 2).

The binding status of the lectins used in the study to cell membranes is briefly shown in Table 2 below.

TABLE 2. Lectin binding of *Proteus* and control groups

| **Lectin** | **The carbohydrate group it binds to** | **Control Group** | ***Proteus* Group** |
|---|---|---|---|
| Con A | α-D- Mannose > α-D-Glucose | (++) | (+++) |
| PNA | β-D Galactose and β-D- N-Acetyl Galactoseamine | (+++) | (++) |
| BPA | β-D Galactose ve β-D D- N-Acetyl Galactoseamine | (+++) | (++) |
| GS-I | α- D- Galactose | (+++) | (++) |
| UEA-I | α-L-Fucose | (+) | (++) |

**+++ Strong, ++ Medium strong, + Less strong**

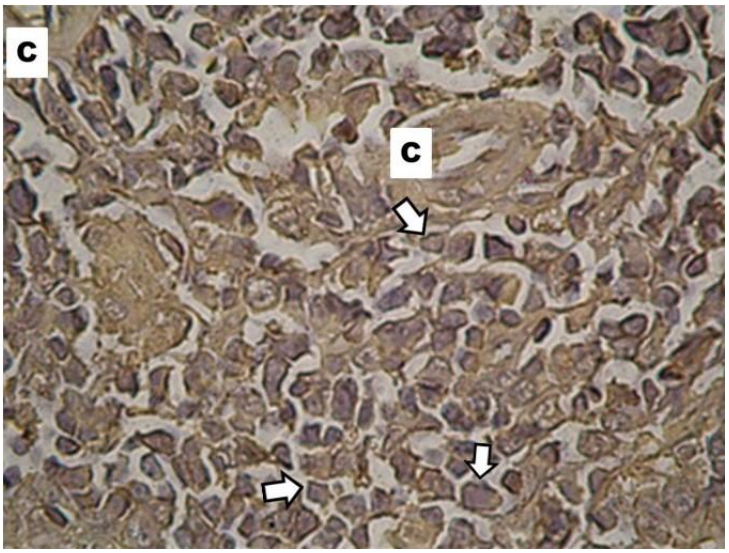


FIGURE 2. Moderate strong (++) staining of control group rabbit spleen cell membranes with Con A. (→) Cell membrane stained with lectin, (C) Capillary. x1000

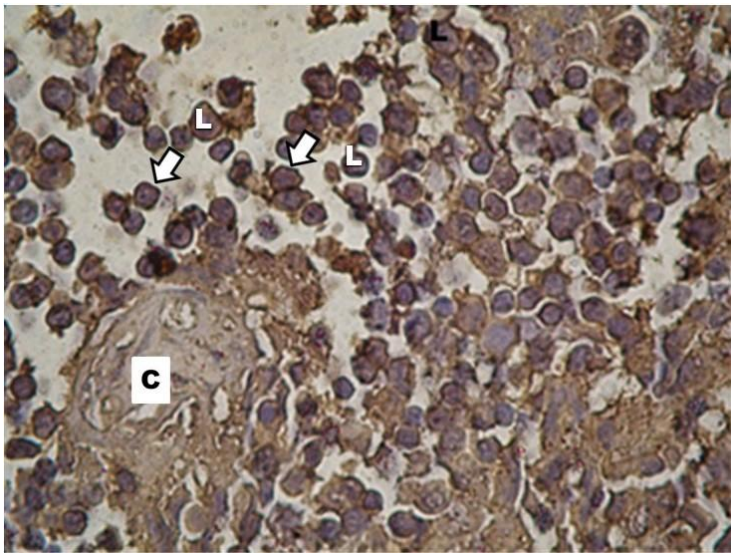


FIGURE 3. Strongly (+++) staining of *Proteus*-treated rabbit spleen cell membranes with Con A. (→) Lectin stained cell membrane, (L) Lymphocyte, (C) Capillary. x1000

PNA, which was used in the study and specifically bound to β-D galactose and β-D N-Acetylgalactosamine, was strongly (+++) bound to the spleen cell membranes of control group rabbits (Figure 3), and to the spleen cell membranes of *Proteus* rabbits. It was observed that it bonded with moderate strong (++) (Figure 4).

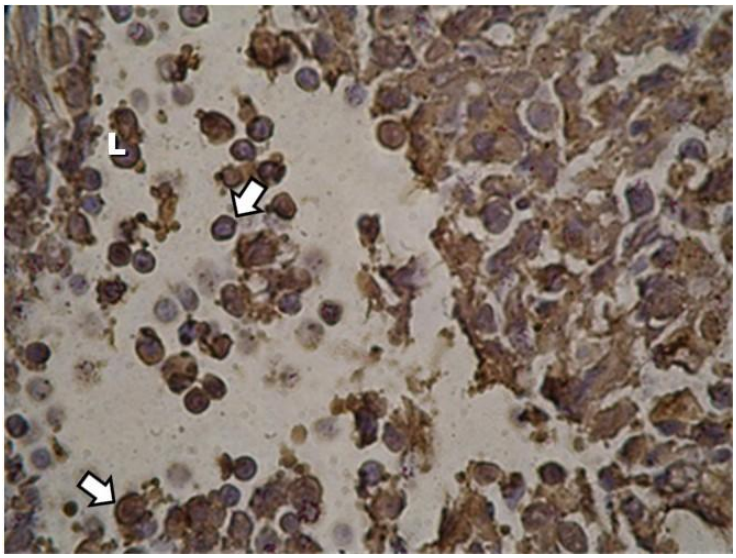


FIGURE 4. Strong (+++) staining of control group rabbit spleen cell membranes with PNA. (→) Lectin stained cell membrane, (L) Lymphocyte. x1000

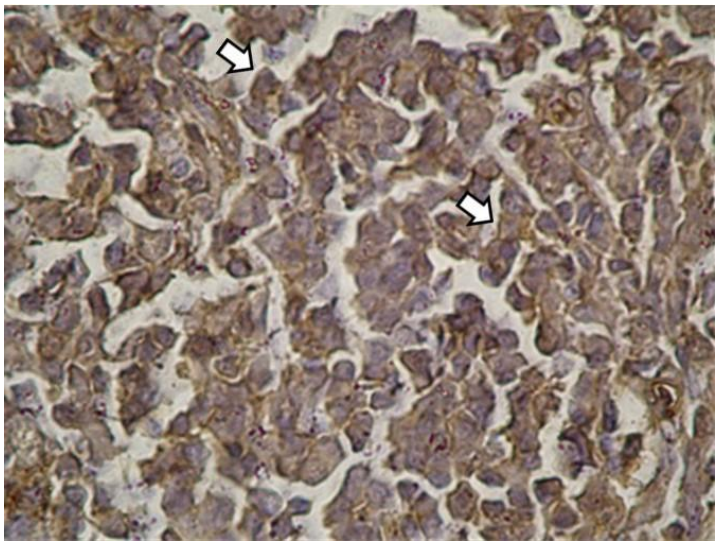

FIGURE 5. Medium strong (++) staining with PNA of rabbit spleen cell membranes given *Proteus vulgaris* OX19 strain. (→) Cell membrane stained with lectin. x1000

BPA, one of the lectins used in the experiments, showed a similar situation to PNA binding. While BPA bound strongly (+++) to the spleen cell membranes of rabbits in the control group (Figure 5), it bound to the spleen cell membranes of rabbits injected with *Proteus* bacteria with moderate strong (++) (Figure 6).

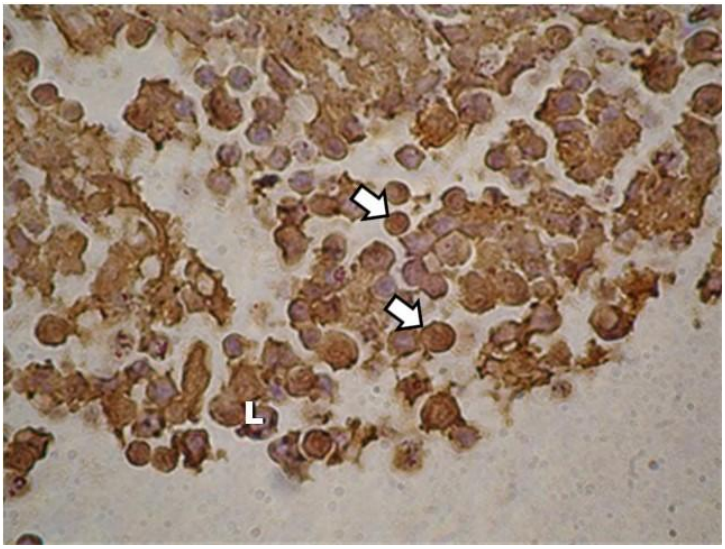


FIGURE 6. Strongly (+++) staining of control group rabbit spleen cell membranes with BPA. (→) Lectin stained cell membrane, (L) Lymphocyte. x1000

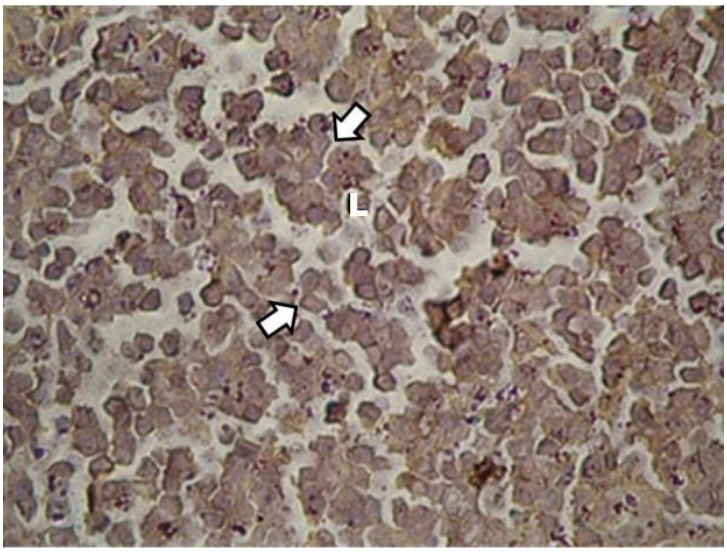


FIGURE 7. Moderate strong (++) staining of rabbit spleen cell membranes in the *Proteus* treated group with BPA. (→) Lectin stained cell membrane, (L) Lymphocyte. x1000

It was observed that BS-I lectin, which specifically binds to α-D-Galactose, bound strongly (+++) to the spleen cell membranes in the control group (Figure 7), and bound to the spleen cell membranes of rabbits in the *Proteus*-injected group with moderate strong (++). (Figure 8).

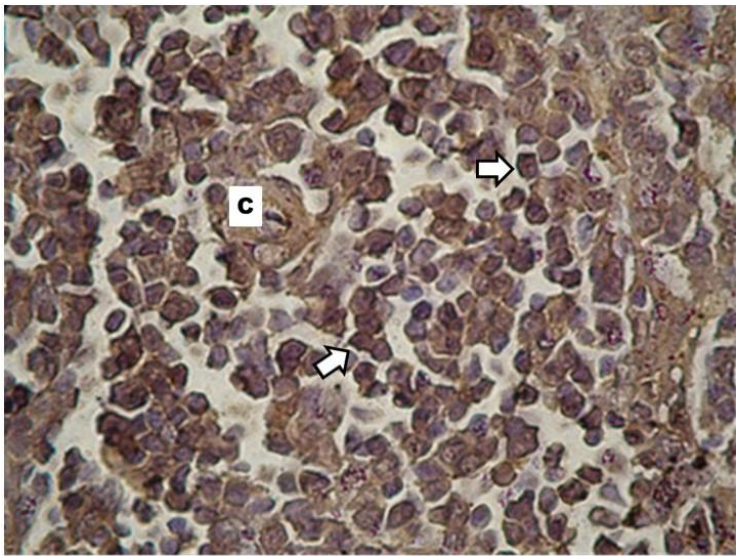


FIGURE 8. Strongly (+++) staining of control group rabbit spleen cell membranes with BS-I. (→) Lectin stained cell membrane, (C) Capillary. x1000

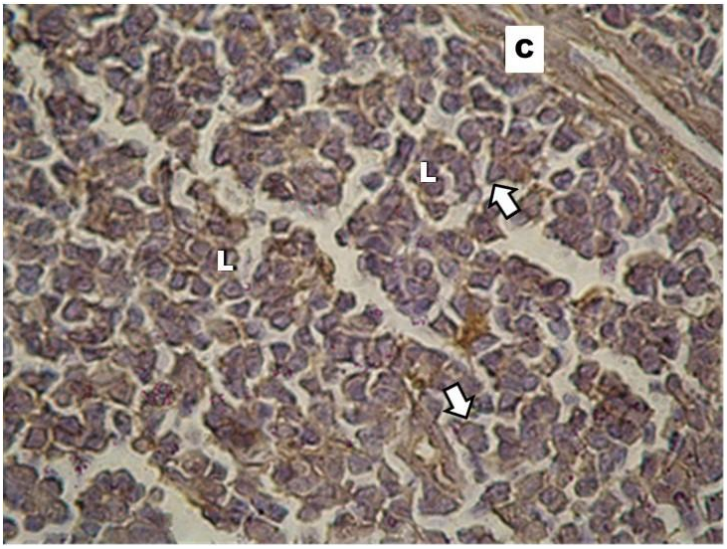


FIGURE 9. Moderate strong (++) staining of Proteus group rabbit spleen cell membranes with BS-I. (→) Cell membrane stained with lectin, (C) Capillary, (L) Lymphocyte. x1000

UEA-I specifically binds to α-L-Fucose (Table 2). It was observed that this lectin bound to the spleen cell membranes in the control group with a weakly strong (+) (Figure 9), and to the *Proteus* cell membranes with a moderate strong (++) (Figure 10).

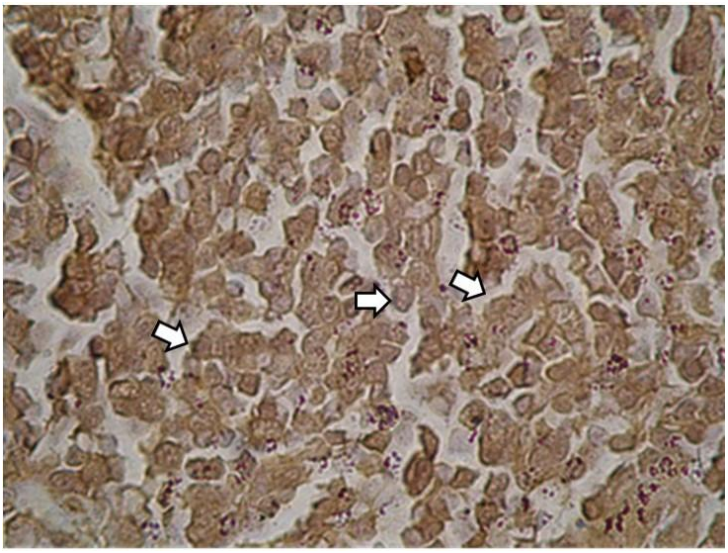

FIGURE 10. Weakly strong (+) staining of control group rabbit spleen cell membranes with UEA. (→) Cell membrane stained with lectin. x1000

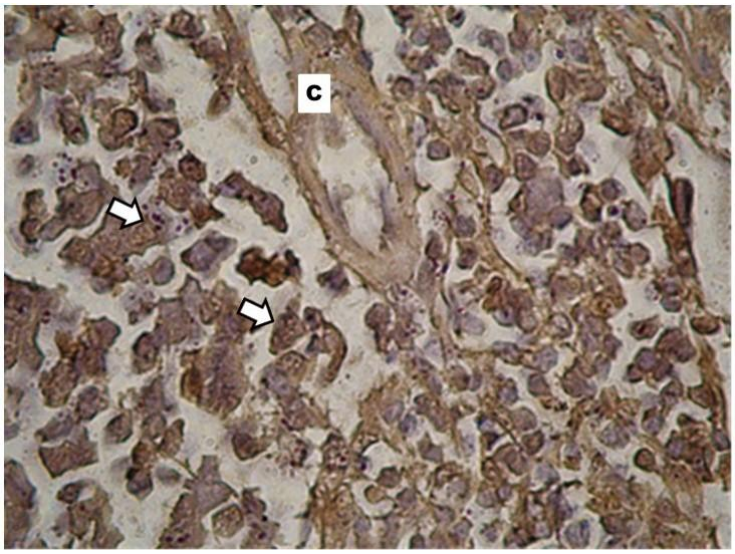


FIGURE 11. Moderate strong (++) staining of rabbit spleen cell membranes injected with *Proteus vulgaris* OX19 strain with UEA-I. (→) Cell membrane stained with lectin, (C) Capillary. x1000

## 4. DISCUSSION

Considering the binding of other lectins used, it was observed that the rabbit spleen cell membranes in the *Proteus* group bound lectins (BPA, PNA and GS-I) less than the control group. BPA, PNA and GS-I lectins bind to D-Galactose units. At the same time, BPA and PNA lectins also bind to β-D N-Acetyl galactosamine [28]. Infected rabbit spleen cell membranes bind less lectins than the control group, suggesting that D-Galactose and β-D N-Acetylgalactosamine units are likely reduced in cell membranes. It was thought that the increase or decrease in carbohydrate units in infected rabbits may have affected the synthesis of glycoproteins containing carbohydrates in the membrane or the enzyme synthesis that binds/separates carbohydrates.

Changes in the carbohydrate expression and glycosylation of cells during the development, diseases and embryogenesis of living things are detected by the binding of lectins to the cells [29, 30]. Sobral et al. [30] reported that changes in cell adhesion, changes in the tumor cell surface and changes in the carbohydrate composition on the surface of the cells occur in metastasis and abnormal cell development. The same researchers examined Con A and UEA-I lectin binding to parotid gland cell membranes with mucoepidermal carcinoma and normal parotid gland cell membranes. Researchers have suggested that Con A binds weakly to normal parotid gland myoepithelial cells and vascular endothelium, but strongly binds to parotid gland duct cells with moderate and high levels of carcinoma. In our study, Con A bound more strongly to the cell membranes of *Proteus* rabbits than the control group. Accordingly, the increase in the binding

of Con A to cancer cell membranes and *Proteus* cells shows that there is an increase in the expression of D-Mannose and D-Glucose in the cell membranes. According to the observations made by some researchers, it has been understood that Mannose units increase in the cell membranes of organisms against urinary tract infection of bacteria [31-33]. It has been reported that mannose found in cell membranes binds to Escherichia coli bacteria, which causes urinary tract infection, and thus is excreted along with the bacteria adhering to D-Mannose during urination [32]. *Proteus* and *E.coli* are in the same family (Enterobacteriaceae), in rabbit spleen cell membranes treated with this bacteria (*Proteus vulgaris* OX19), Con A (which specifically binds mannose) increased due to infection, thus mannose on the cell surfaces may have increased. It is estimated.

It has been reported that parasitic organisms cause changes in the cell membranes of the host organism. Melo-Júnior et al. [34] observed under light microscopy the binding of lectins to cells in hepatic-egg granuloma caused by Schistoma mansoni in humans. It has been reported that Con A and PNA lectins bind moderately strongly (++) to normal hepatic cells, while PNA binds strongly (+++) to cells in hepatic-egg granuloma and Con A binds weakly. Lectin binding to cell membranes in *Proteus* rabbits was different from the lectin binding detected by Melo-Júnior et al. For example, although Con A bound strongly to the cells of *Proteus* rabbits compared to the control group, Con A bound weakly to the cells in the hepatic-egg granuloma. PNA binding also showed a different situation. Although PNA binds moderately to *proteus* cells, it has been reported that PNA binds very strongly to cells in hepatic-egg granuloma. Tritrichomonas foetus was inoculated into the genital organs of mice, and genital tissue was taken from the mice at intervals from the 3rd day to 60 days. It has been reported that the binding of PNA and SBA lectins to uterine endometrial cells is significantly different compared to the infected group. It was reported that 16 days after parasite application, UEA-I bound strongly to the vaginal epithelium, while PNA and SBA lectins bound strongly to uterine cells [35]. In our study, although PNA and UEA-I were bound to a moderate degree in *Proteus* spleen samples, PNA was bound to a high degree and UEA-I to a low degree in the control group.

Alroy et al. [36] investigated the binding of Con A, UEA-I and PNA lectins to spleen cells in the diagnosis of carbohydrate storage diseases. It has been observed that in mannose storage disease (mannosidosis), Con A binds very strongly to spleen cells, while UEA-I and PNA do not bind at all. It has been reported that in fucose storage disease (fucosidosis), Con A binds weakly, UEA-I binds moderately, and PNA does not bind at all. Spleen cells in mannosidosis showed strong binding of Con A and low binding of PNA, similar to spleen cells with *Proteus*. It is thought that lectins bind with different strengths to spleen cells in carbohydrate storage disease and to spleen cells infected with *Proteus*, and disease and infection may affect the structural and functional properties of enzymes that bind or degrade carbohydrates.

Considering the histochemical studies conducted with lectins, it is thought that in infectious conditions and cancer, a change occurs in the form of an increase

or decrease in the carbohydrates at the ends of the glycoproteins or glycolipids in the cell membranes, compared to normal or control group experimental animals.

**Acknowledgement** This study was produced and developed from Maryam Diani's master thesis.

**Author Contribution Statements** MD and MNA: designed the study, performed the wet-lab work and analyzed the data, wrote and reviewed the manuscript. HE and NG: writing–review & editing.

**Declaration of Competing Interests** The authors declare no conflict of interest.

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