Differential Expression and Localisation of Acetyl-Glucosamine, Acetyl-Galactosamine, Galactose, Mannose and Glucose Specific Lectins in Lingual Tonsil of Buffalo (Bubalus bubalis)

Pawan Kumar*

Department of Veterinary Anatomy, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India

Abstract: The present study is the most comprehensive representation of lectin binding sites in various structural components of the lingual tonsil of six adult healthy buffaloes. The study was useful in determining the specific binding affinities of sixteen lectins of the N-acetylglucosamine group, i.e., *Triticum vulgaris* (WGA), succinylated *Triticum vulgaris* (s-WGA), *Lycopersicon esculentum* (LEL), *Datura stramonium* (DSL), *Solanum tuberosum* (STL); N-acetylgalactosamine group, i.e., *Glycine max* (SBA), *Dolichos biflorus* (DBA), *Ricinus communis* (RCA), *Vicia villosa* agglutinin (VVL); galactose group i. e *Griffonia simplicifolia* isolectin B4 (GS1B4), *Arachis hypogaea* (PNA), *Artocarpus integrifolia* (Jacalin), *Erythrina crisa-galli* (ECL); and glucose/mannose group i.e. *Canavalia ensiformis* (Con A), *Lens culinaris* agglutinin (LCA), *Pisum sativum* agglutinin (PSA) at structures of the lingual tonsil. The stratum spinosum of stratified squamous epithelium and modified reticular epithelium was strongly demarcated by lectins of the N-acetylglucosamine group. In addition, VVL, jacalin, and Con A lectins also showed strong responses for the same layer. The RCA, PNA, Con A, and PSA were the best markers for the collagen fibers of the subepithelial connective tissue. The lymphoid cells of the inter- and parafollicular region possessed receptors for ECL, WGA, and Con A. In contrast, the germinal center B cells were labeled only by the lectins of the glucose/mannose group. The mucosal secretions and the endothelium of the blood vessels were predominantly composed of glucosamine, sialic acid, and galactosamine sugars. The characteristic localization of lectins suggests the presence of specific receptor sites that may be useful for studying early disease pathogenesis and developing oral vaccines. In addition, the study will provide a database for comparing histochemical changes in different disease states.

Keywords: Buffalo, lectins, lingual tonsil, lymphoid tissue, reticular epithelium.

INTRODUCTION

The light and ultrastructure of the lingual tonsils of buffaloes have been studied in detail [1, 2]. The tonsils, the mucosa-associated lymphoid tissue, act as ports of entry for various pathogens and are involved in the uptake of antigens from the mucosal immune system, especially towards the reticular epithelium. The lingual tonsil has a stratified, keratinized squamous epithelium on the free surface that transforms into a non-keratinized, reticular epithelium due to infiltration of the underlying lymphoid tissue, and it is vulnerable to a variety of harsh feeds and constant exposure to antigens. The mucous secretions of the glandular tissue were strongly positive for acidic and neutral mucopolysaccharides, mucins, glycogen, weakly sulfated mucopolysaccharides, hyaluronic acid, and sialomucins [1].

Lectins have been used as a tool for histochemical qualitative detection of glycoconjugate expression of various sugars at different tissue or organ sites [3, 4]. Lectins are proteins or glycoproteins with specific affinity for carbohydrate moieties of glycoconjugates, which are the major components of the glycocalyx of many cell types and are involved in osmoregulation, cell-to-cell recognition, hormone binding, protection of the cell from phagocytosis and desiccation, differentiation, defense, and ion transport [5]. Different cell types in their normal state of health and, as they transform into diseased cells, express different glycan arrangements, and this specificity can be exploited by using lectins as carrier molecules for targeted drug delivery [6]. Furthermore, this receptor-mediated bio-adhesion can be used to trigger vesicular transport into or through polarised epithelial cells [7]. The conjugation of drug formulations to lectins as carrier molecules or vice-versa has enhanced drug delivery to a particular group of cells or systemic circulation, as demonstrated in *in vitro* models [8]. Lectin-containing delivery systems, especially employing endogenous lectins to act as 'handles,' are a potential innovation for targeted and prolonged therapy within the mouth cavity [9].

A large amount of literature is available on the structure of the tonsils, but only a few passing references are available on lectin histochemistry [10, 11]. The present study was planned to exhibit the localization of various sites in structural components of the tonsils by different categories of lectins that might be utilized for immunological studies by the researchers. The study's results will characterize

*Address correspondence to this author at the Department of Veterinary Anatomy, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India; Tel: +919466402637; E-mail: pawanrajoria2000@rediffmail.com

ISSN: 1927-5196 / E-ISSN: 1927-520X/23

different sugar moieties present in the secretions with probable different functional significance. This will generate new information because of the very first extensive lectin histochemical study on the tonsil of buffalo amongst domestic animals.

MATERIALS AND METHODS

The present study was performed on six clinically healthy adult buffaloes (Bubalus bubalis), aged 5-6 years, of a local mixed breed regardless of sex. The heads were procured from Ghazipur Municipal Slaughterhouse, New Delhi, India, immediately after their killing by the bolt gun method. There was no need for approval from the Institutional Animal Ethical Committee as the tissues were collected from the slaughterhouse. Tissue was removed from the lingual tonsils and preserved in liquid nitrogen. Small pieces of the frozen tonsils were embedded in tissue freezing medium to make blocks, and frozen sections of 6-8 µ cut with a cryostat were collected on glass slides treated with a 2% solution of 3-aminopropyltriethoxy-silane and stored in a freezer at -20 °C. The slides were thawed and air-dried at room temperature for 30 minutes. The sections were fixed in acetone for 20 minutes at room temperature, air dried, and rehydrated in phosphate buffer saline (PBS) pH 7.4 for 10 minutes. The frozen sections were rimmed with a PAP pen (Sigma) to save the chemicals. The sections were treated with 0.3% H_2O_2 in PBS for 30 minutes to block endogenous peroxidase activity, washing in PBS,

Table 1: Lectins used for the Present Study with Specific Sugar Moieties

Lectin	Common Name	Acronym	Major sugar moieties/ specificity
N-acetylglucosamine group			
Triticum vulgaris	Wheat germ	WGA	N-acetyl-D-glucosamine and Sialic acid (sia) (β-GlcNAc)
Succinylated *Triticum vulgaris*	Wheat germ, succinylated	s-WGA	N-acetylglucosamine (β-GlcNAc)
Lycopersicon esculentum	Tomato lectin	LEL	[GlcNAc]1-3, N-Acetylglucosamine
Datura stramonium	Datura	DSL	[GlcNAc]1-3, N-Acetylglucosamine
Solanum tuberosum	Potato lectin	STL	N-Acetylglucosamine (β-GlcNAc)
N-acetylgalactosamine group			
Glycine max	Soybean	SBA	N-acetylgalactosamine
Dolichos biflorus	Horse gram	DBA	α-linked N-acetylgalactosamine (α-GalNAc)
Ricinus communis	Castor bean	RCA	N-acetylgalactosamine
Vicia villosa agglutinin	Hairy vetch	VVL	α- or β-linked terminal N-acetylgalactosamine
Galactose group			
Griffonia simplicifolia isolectin B4	Africa shrub legume	GS1B4	Terminal (1-3)-linked galactose epitopes
Arachis hypogaea	Peanut agglutinin	PNA	Galactose-β(1-3) N-acetylgalactosamine Gal-β(1-3)-GalNAc
Artocarpus integrifolia	Jackfruit	Jacalin	Galactose, β (1,3) N-aetylgalactosamine
Erythrina crisagalli	Coral tree	ECL	Galactose, N-acetylgalactosamine, Lactose β -Gal>β-GalNAc
Glucose/Mannose group			
Canavalia ensiformis Concanavalin A	Jack bean	Con A	Terminal α-D-mannosyl and α-D-glucosyl groups *α-Man>α-Glc*
Lens culinaris agglutinin	Common lentil	LCA	α-linked Mannose, Glucose specific for *α-Man>α-Glc*
Pisum sativum agglutinin	Pea	PSA	α-linked Mannose, Glucose *specific for α-Man>α-Glc*

Gal: Galactose, GalNAc: N-acetyl-D-galactosamine, GlcNAc: N-acetyl-D-glucosamine, Sia: Sialic acid (N-acetylneuraminic acid).

followed by 1% bovine serum albumin (BSA) in PBS for 45 minutes to block the binding of nonspecific antigens. Separate sections were incubated with biotinylated lectins (Table **1**) purchased from Vector Lab. at a concentration of 10µg/ml in 0.2% gelatin-PBS for 1 hour in a humidification chamber at room temperature. After washing three times with PBS (5 minutes each), the sections were incubated with streptavidin, Alexa Fluor™ 488 conjugate (2 µg/ml in PBS) for 30 minutes and washed again twice in distilled water (5 minutes each). Sections were embedded with coverslips in Mowiol containing 2.5% 1, 4-diazabicyclo [2.2.2] octane (DABCO) and viewed under a fluorescence microscope to capture micrographs. Control tonsil tissue sections were treated as described in the protocol above, except for the incubation step with biotinylated lectin to eliminate the possibility of nonspecific binding.

RESULTS

The lingual tonsil of buffaloes was lined by stratified squamous epithelium, keratinized to non-keratinized, with a varying number of rows in different layers. The epithelium toward the crypts was transformed into reticular epithelium in the region of the lymphoid tissue. The propria submucosa exhibited loose, irregular connective tissue, lymphatic tissue, blood vessels, and mucous glands. Frozen sections treated for lectin histochemistry yielded the following observations:

N-Acetylglucosamine Group

The five lectins of the N-acetylglucosamine group (WGA, s-WGA, LEL, DSL, and STL) showed a positive affinity for the majority of cells of the stratum basale. The intensity of the response was dramatically increased in the cells of the stratum spinosum (Figure **1A-D**). However, the reactivity of the lectins gradually decreased toward the superficial layers of the stratum granulosum. Eventually, the remaining cells of the stratum granulosum no longer showed a positive response to these lectins. The stratum corneum showed no positive affinity for any of the lectins of the N-acetylglucosamine group; however, the most superficial keratinized cell layers showed a positive response for these lectins (Figure **1D**). The reticular epithelium also responded strongly to these lectins. The connective tissue of the propria submucosa, which consists mainly of collagen and reticular fibers, also showed a moderate positive affinity for these lectins, with the exception of s-WGA, which showed a comparatively weaker response. The various components of the lymphoid tissue were negative for binding of the lectins, with the exception of some

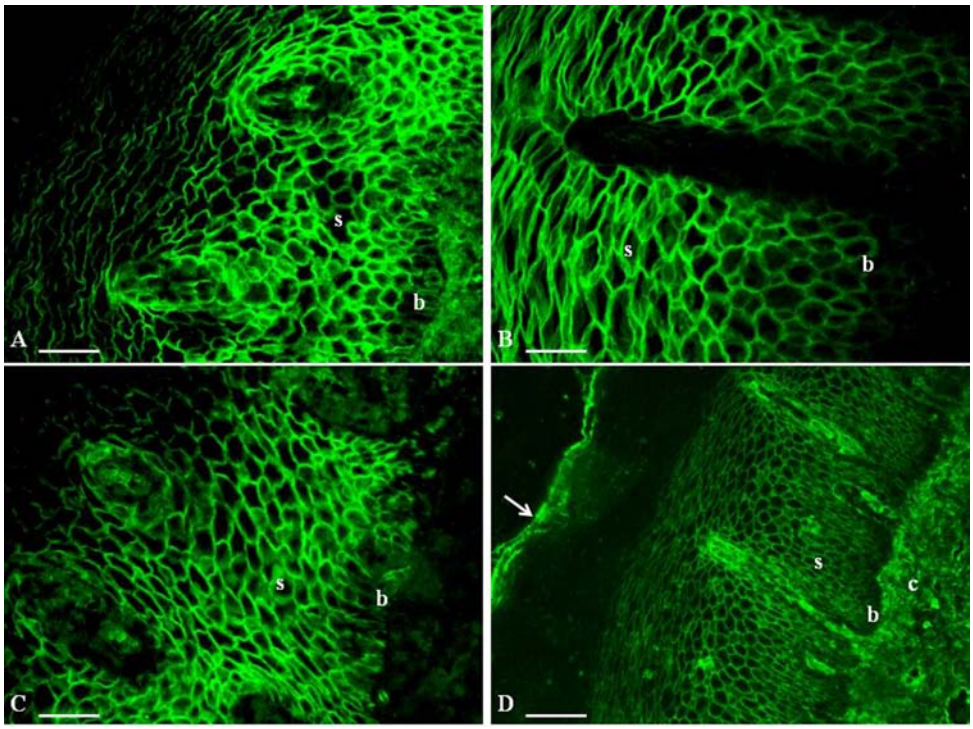

Figure 1: Photomicrograph showing the presence of lectins of the N-acetylglucosamine group in the lingual tonsil of the buffalo. A strong positive reaction in the stratum spinosum (S), weak in stratum basale (b), and absence in the other strata. **A.** WGA x 200 (bar 100 µm); **B.** s-WGA x 400 (bar 50 µm); **C.** DSL x 200 (bar 100 µm); **D.** In addition, note presence of lectin in the most superficial layers (arrow) and subepithelial connective tissue (c). STL x 100 (bar 200 µm).

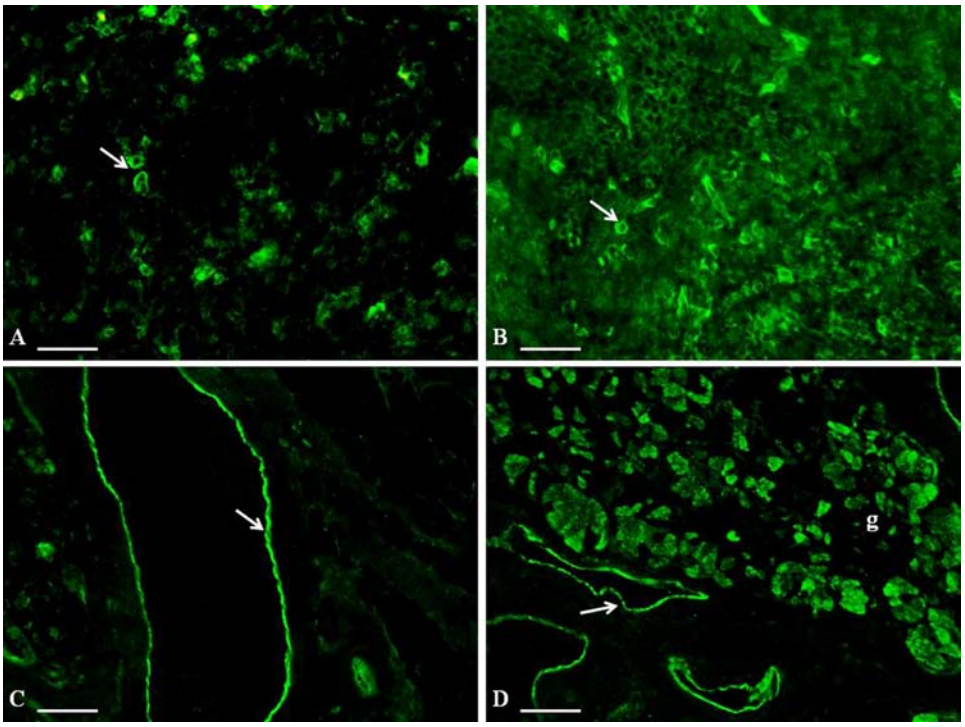

Figure 2: Photomicrograph showing the presence of lectins of the N-acetylglucosamine group in the lingual tonsil of the buffalo. **A.** Presence of WGA in some of the lymphoid cells (arrow) of interfollicular area. x 200 (bar 100 µm); **B.** A weak to moderate reaction in the lymphocytes except for a few strongly positive lymphoid cells (arrow). LEL x 200 (bar 100 µm); **C.** Endothelium of the blood vessels (arrow) having a strong affinity for DSL x 100 (bar 200 µm); **D.** Note the presence of varying concentrations of LEL in the endothelium of the blood vessels (arrow) and glandular acini (g). x 100 (bar 200 µm).

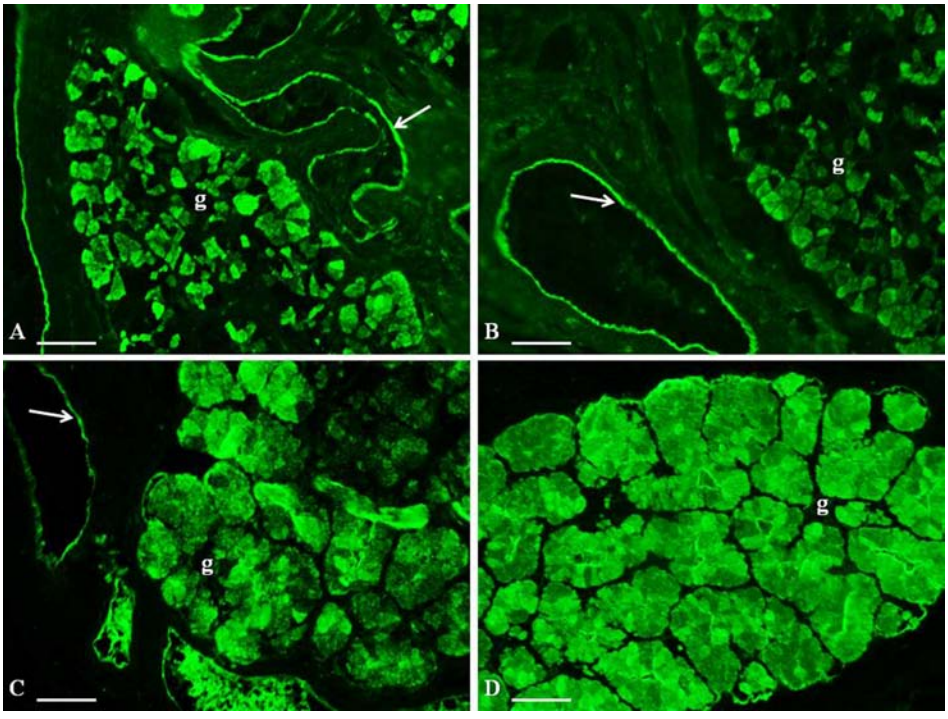

Figure 3: Photomicrograph showing the presence of GlcNAc lectins in the lingual tonsil of the buffalo. **A.** A positive reaction in the glandular acini (g) and endothelium of the blood vessels (arrow). STL x 100 (bar 100 µm); **B.** WGA x 100 (bar 100 µm); **C.** Note comparatively stronger reaction in the glandular acini(g). s-WGA x 100 (bar 200 µm); **D.** A higher magnification of glandular acini (g) showing varying reactions in different cells and luminal surfaces. s-WGA x 200 (bar 100 µm).

Table 2: Specificity of Different Lectins in Structural Components of the Lingual Tonsil of Buffalo

N-acetyl glucosamine group									
Lectin	**Epithelium**					**CT**	**LT**	**GL**	**EN**
	SB	**SS**	**SG**	**SC**	**KL**				
WGA	+, ±	+++	±	_	++	++	± Few ++*	+, ±	++
s-WGA	+, ±	+++	±	_	++, RE ++	±	-	+++, ±	++
LEL	+, ±	+++	±	_	++, RE ++	++	+ Few +*	++, ±	+++
DSL	+, ±	+++	±	_	++, RE -	+	-	+, ±	+++
STL	+, ±	+++	±	_	++, RE -	++	+ Few +*	++, ±	+++
N-acetyl galactosamine group									
SBA	±	±	-	-	++	±	-	++, ±	++
DBA	±	±	-	-	++	±	-	++, ±	++
RCA	±	±	-	-	+, RE ±	+++	-	-, ±	+++
VVL	+, ±	+++	-	-	++, RE ±	±	-	++, ±	+++
Galactose group									
GS1B4	±	++	-	-	++ RE +++	+	+*	++	++
PNA	±	±	-	-	- RE±	+++	±*	-, ±	±
Jacalin	+	+++	-, ±	-	++ RE±	+	++*	+++	++
ECL	±	±	-	-	++ RE±	++	+++* +**	-, ±	++
Glucose/Mannose group									
Con A	++	+++	++, ±	-	++ RE-	+++	++* ++**	-, ±	++
PSA	±	+	--	-	+++ RE-	+++	+* +**	-, ±	+
LCA	+, ±	++	+, ±	-	++ RE-	++	+* +**	±, ++	+

SB-stratum basale; SS-stratum spinosum; SG-stratum granulosum; SC-stratum corneum; KL-keratinised layer; RE-reticular epithelium; CT-connective tissue; LT-lymphoid tissue; GL-glands; EN-endothelium.
± weak to absent, + weak, ++ moderate, +++ strong, * inter and parafollicular, ** germinal centre.

isolated lymphoid cells and probably macrophages in the interfollicular areas (Figure **2A**). The lectin LEL additionally showed an affinity for lymphocytes in the interfollicular region (Figure **2B**), whereas the DSL showed no positivity for any type of lymphoid cell. A strong positive response was observed at the luminal surface of blood vessels belonging to the endothelium (Figures **2C, D**, and **3A, B**), with WGA and s-WGA lectins showing comparatively lower positivity (Figure **3C**). The mucous acini were positive for all of these lectins, but the localization pattern was different for the different lectins in the group (Figures **2D** and **3A-D**). Some of the glandular lobules had strongly positive acini, whereas the others were completely devoid of positive reaction. A few lobes had a mixed distribution of acini, showing both the presence and absence of activity. A comparatively higher lectin concentration was observed on the luminal surface of the glandular acini. Most of the glandular ducts showed no positive response, except for the interlobular ducts, which showed a strong positive response in the form of a plexus toward their lumen (Figure **3C**). Increased affinity for the lectin s-WGA was observed in all of the above glandular components (Figure **3C, D**). The

negative control sections showed no fluorescence, indicating that there was no false-positive reaction. The positive responses in different components of the tonsils were qualitatively shown (Table **2**).

N-Acetylgalactosamine Group

The N-acetylgalactosamine group (SBA, DBA, RCA, and VVL) of lectins showed weak localization in the strata basale and spinosum, absence in the stratum granulosum, and moderate in the most superficial cells of the keratinized layer, with the exception of VVL, which showed a positive response similar to that of the N-acetylglucosamine group (Figure **4A, B**). These lectins bind only weakly to the reticular epithelium (Figure **4C**). The connective tissue, especially in the subepithelial area and at the periphery of the glandular acini, was very strongly positive for the lectin RCA. The connective tissue for the other lectins in the group showed a weaker response. These lectins showed no positive affinity for any population of lymphoid cells. The endothelium of the various types of blood vessels showed a strong positive response for the lectins RCA and VVL and a comparatively weaker response for the lectins SBA and DBA. The lectins in this group occurred moderately to strongly in some of the glandular acini, with the exception of RCA, where no positivity was observed. The distribution pattern of these lectins was also similar to that of the previous group; that is, positivity was not localized in all glandular acini (Figures **4D** and **5A, B**).

Galactose Group

The lectins of the galactose group (GS1B4, PNA, jacalin, and ECL) reacted less strongly to the different layers of the stratified squamous epithelium, except for the lectins jacalin and GS1B4, which showed a strong positive reaction in the stratum spinosum and a moderate reaction in the stratum basale (Figure **5C, D**). All lectins showed a weak to moderate positive response in the keratinized layer except for the lectin jacalin, which showed greater affinity (Figure **6A**). These lectins showed a weak response in the reticular epithelium with the exception of the lectin GS1B4, which showed moderate to strong positivity (Figure **6B, C**). Connective tissue was positive for all of these lectins, especially PNA, which showed strong affinity in the subepithelial area where collagen fibers predominate (Figures **5C** and **6C**). The galactose group

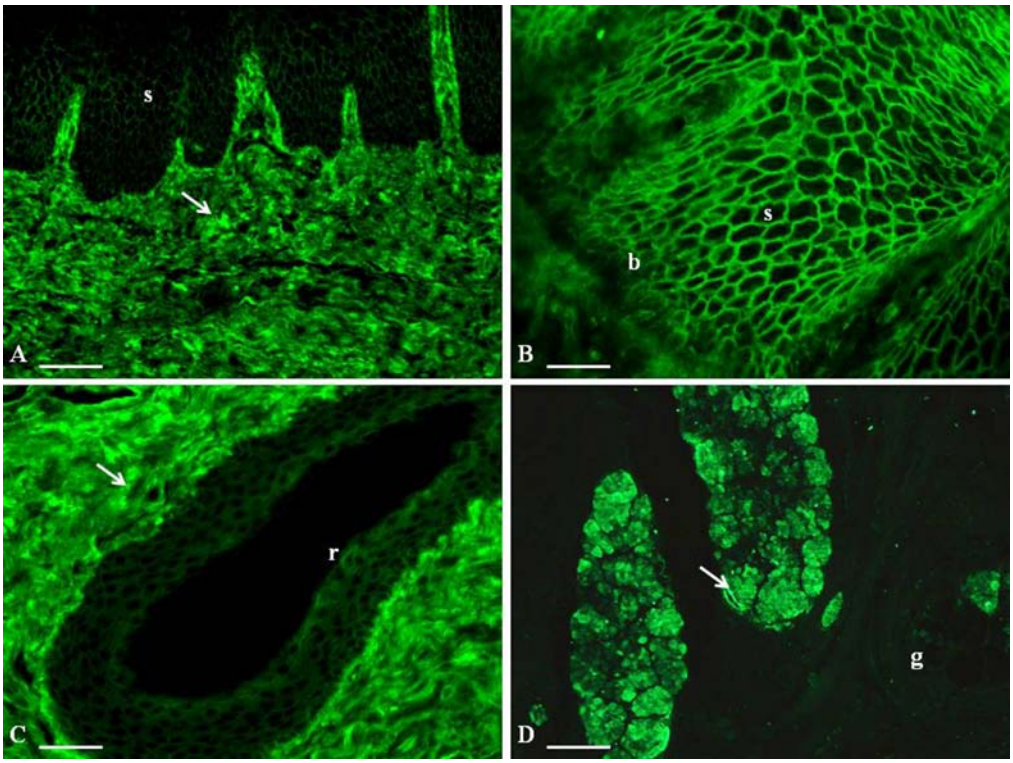

Figure 4: Photomicrograph showing the presence of N-acetylgalactosamine group lectins in the lingual tonsil of the buffalo. **A.** A very weak negligible reaction to the stratum spinosum layer (s) of the epithelium. Not a strong positive activity in the subepithelial connective tissue having collagen fibers (arrow). RCA x 200 (bar 100 µm); **B.** Strong localization of VVL lectin in the stratum spinosum (s) and very weak in the stratum basale (b) layer. X 200 (bar 100 µm); **C.** A weak and strong reactivity of the lectin RCA in the reticular epithelium (r) and the subepithelial connective tissue (arrow), respectively. x 200 (bar 100 µm); **D.** Mucous acini showing negative reaction except for a few clusters (g) having strong affinity (arrow) for SBA. x 200 (bar 100 µm).

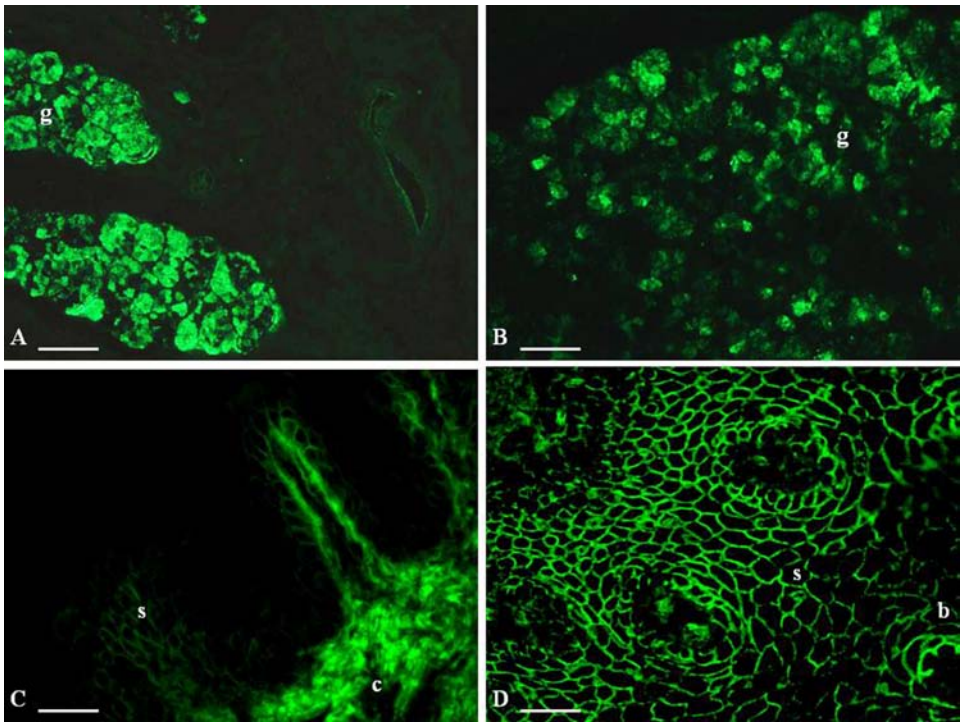

Figure 5: Photomicrograph showing the presence of N-acetylgalactosamine and galactose groups of lectins in the lingual tonsil of the buffalo. **A.** The mucous glandular acini (g) shows the presence of lectin DBA. x 200; **B.** VVL x 200; **C.** Strong activity of PNA (galactose group lectin) in the subepithelial connective tissue (c) and a very weak reaction in the lower strata (s) of epithelial cells. x 200; **D.** A mild to strong and a weak positivity in the stratum spinosum (s) and stratum basale (b) of the keratinized epithelium. Jacalin x 200.

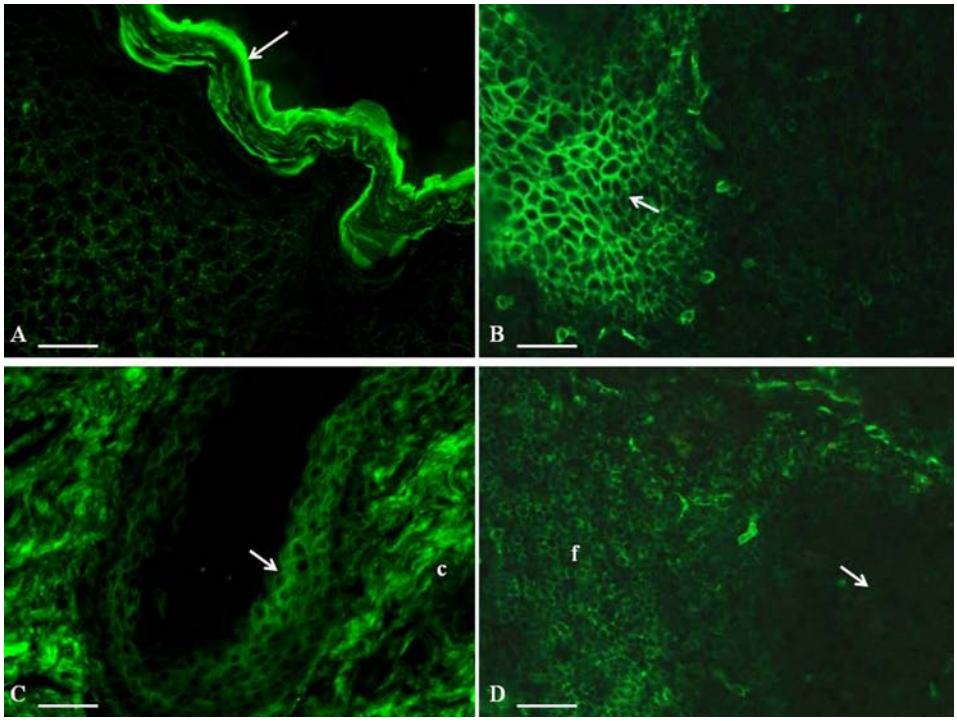

Figure 6: Photomicrograph showing the presence of galactose group of lectins in the lingual tonsil of the buffalo. **A.** A mild to strong positivity in the most superficial layers (arrow) of the keratinized epithelium. Jacalin x 200 (bar 100 µm). **B.** Reticular epithelium (arrow) showing a strong positivity for the lectin GS1 B4. x 200 (bar 100 µm); **C.** A weak and strong binding affinity for the reticular epithelium (arrow) and the subepithelial connective tissue (c), respectively. PNA x 200 (bar 100 µm); **D.** A weak to moderate positive reaction of GS1 B4 for the lymphoid cells of the interfollicular area (f). Note the absence of reaction in the germinal center (arrow) of the lymphoid follicle. x 200 (bar 100 µm).

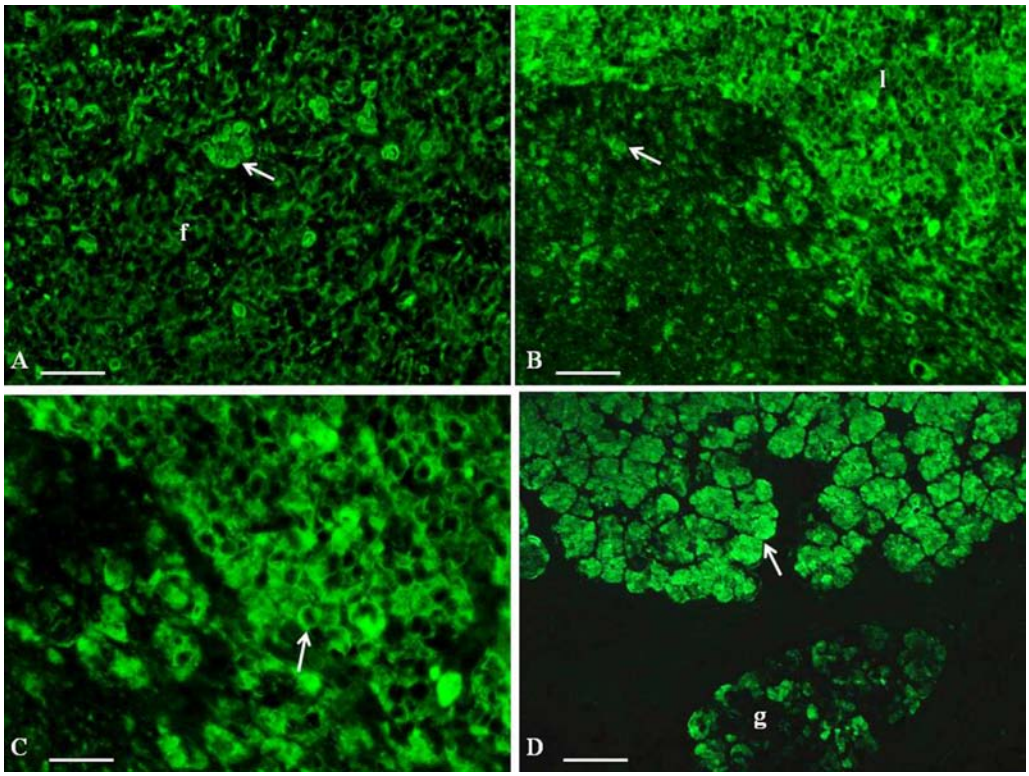

Figure 7: Photomicrograph showing the presence of galactose group of lectins in the lingual tonsil of the buffalo. **A.** The lymphoid cells of the interfollicular area (f) and RBCs of blood vessels (arrow) show a moderate reaction to jacalin. x 200 (bar 100 µm); **B.** A strong positive affinity of ECL for the lymphoid cells (l) of the interfollicular area. Also, note the presence of moderate reactivity in the lymphoid cells of the germinal center (arrow). x 200 (bar 100 µm); **C.** Higher magnification shows the presence of galactose receptors on the surface of the lymphoid cells (arrow). ECL x 400 (bar 50 µm); **D.** A strongly positive reaction of GS1B4 in some of the glandular acini (arrow). Note a weak absence of positivity in the rest of acini (g) x 200 (bar 100 µm).

of lectins also responded to different cell populations of lymphoid tissue. The lectins GS1B4 and jacalin are weakly bound to the lymphoid cells of the interfollicular areas without showing an affinity for the germinal center (Figures **6D** and **7A**). However, the lectin ECL showed a strong affinity for the lymphoid cells of the interfollicular areas and a weaker response for the lymphocytes of the germinal center (Figure **7B, C**). In addition, a few germinal center cells were also moderately positive for ECL. The affinity for the lymphocytes was least pronounced for the lectin PNA. Blood vessels of different sizes showed positive activity toward their endothelium. The secretions of the glands did not contain mucopolysaccharides of the galactose group, with the exception of the lectins GS1B4 and jacalin. The latter showed a generally positive reaction in the majority of the acini, whereas GS1B4 showed strong positivity in some of the glandular acini (Figure **7D**). The peripheral part of the glandular acini in the region of the myoepithelial cells was weakly to moderately reactive to these lectins. The lectin PNA was the only one that showed very strong positive activity in muscle tissue.

Mannose/Glucose Group

The lectins of the mannose/glucose group (Con A, LCA, and PSA) showed binding affinity in different layers of the epithelium, increasing from basal cells to spinosum cells and showing a similar pattern in the most superficial cells (Figure **8A-D**). However, no positivity was observed in the cells of the stratum granulosum, with the exception of lectin Con A, which showed a weak to moderate granular response (Figure **8B**). The subepithelial connective tissue showed similar positivity to the cells of the stratum spinosum (Figure **8C, D**). The connective tissue was strongly positive for the lectins Con A and PSA compared with the LCA lectin of the same group. These lectins labeled the lymphoid cells of the germinal center, but the lymphoid cells, especially in the parafollicular and interfollicular areas, were also strongly positive (Figure **9A, B**). The connective tissue surrounding the blood vessels and the endothelium were weakly and marginally positive, respectively. The secretions of the mucous glands had a comparatively lower concentration of mannose and/or glucose, which was reflected in a weaker reaction (Figure **9C**). However, the positive reaction for the

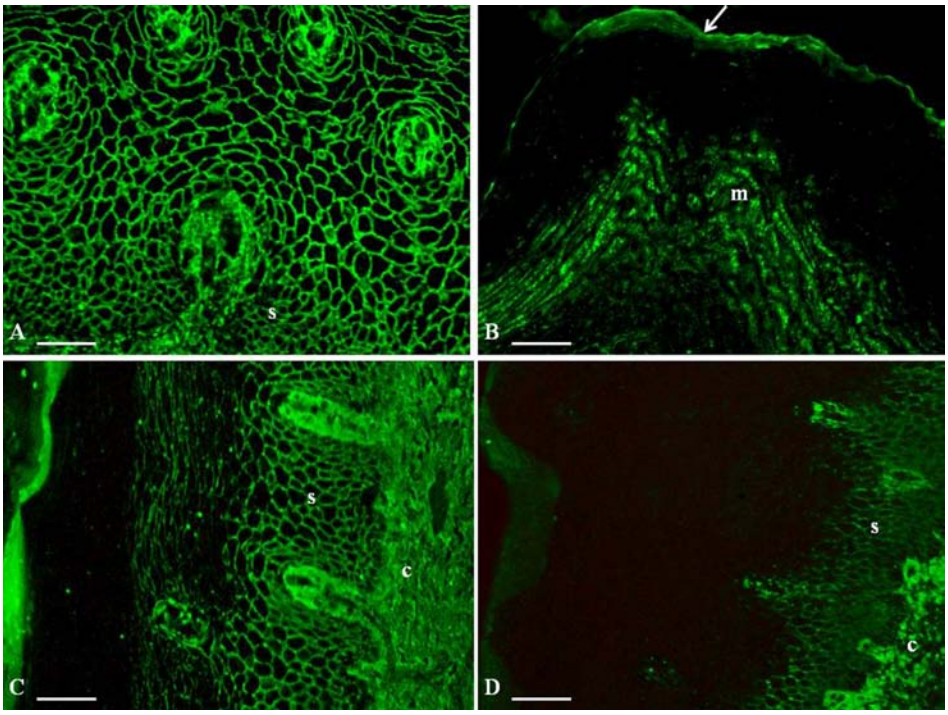

Figure 8: Photomicrograph showing the presence of mannose/glucose group of lectins in the lingual tonsil of the buffalo. **A.** Strata basale and spinosum (s) showing a strong positive reaction for the lectin Con A. x 200 (bar 100 µm); **B.** A moderate reaction in the stratum granulosum (m) and most superficial layers (arrow) of the stratified squamous keratinized epithelium. Con A x 200 (bar 100 µm); **C.** The stratum spinosum (s) showed a positive reaction similar to that of ConA. Note a strong affinity of the collagen fibers (c) for the lectin LCA. x 200 (bar 100 µm); **D.** A weak and a strong reaction exhibited by lectin PSA in the stratum spinosum (s) and subepithelial connective tissue (c), respectively. x 200 (bar 100 µm).

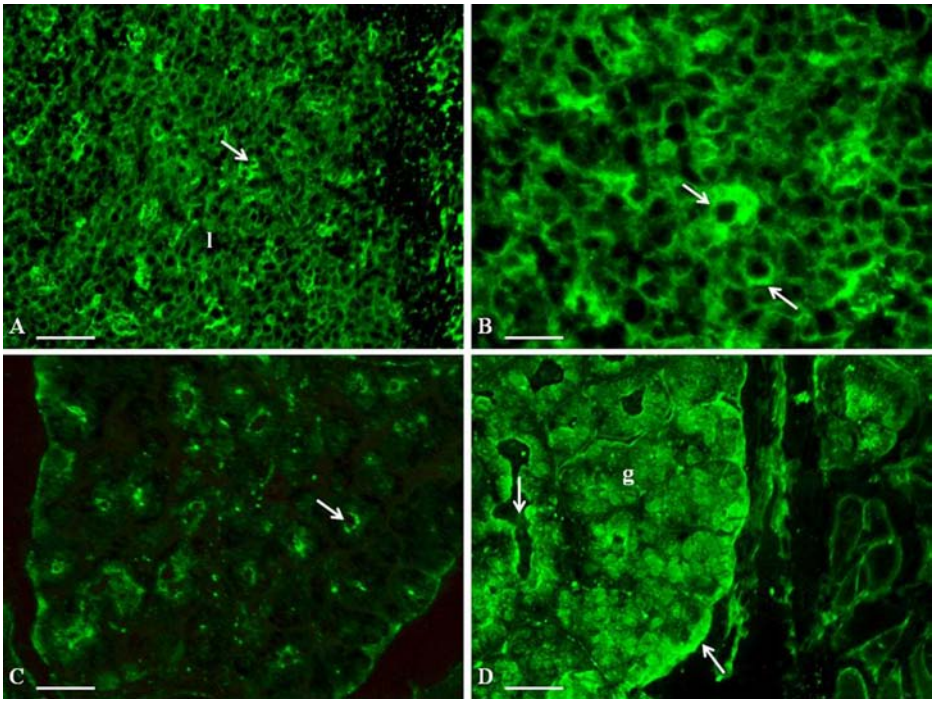

Figure 9: Photomicrograph showing the presence of mannose/glucose group of lectins in the lingual tonsil of the buffalo. **A.** The lymphoid cells of the germinal center (l) showing a positive affinity for the Con A. Note some of the cells (arrow) show a strong positive reaction. x 200 (bar 100 µm); **B.** Lymphoid cells (arrow) showing a pattern of positivity at higher magnification. Con A x 400 (bar 50 µm); **C.** The glandular acini show the absence of mannose/glucose in the secretions except for a weak reaction towards the luminal surface (arrow). PSA x 200 (bar 100 µm); **D.** A moderate positive reaction of lectin LCA in the mucous glandular acini (g). Note a strong reaction towards the periphery and luminal surface (arrow) of the acini and ducts. x 200 (bar 100 µm).

lectin LCA was moderate and comparatively stronger than for the other two lectins in the same group, especially at the luminal border of the glandular acini and ducts (Figure **9D**). These lectins also had a moderate affinity for the myoepithelial cells at the periphery of the glandular acini. The staining pattern was generally stronger for Con A, followed by LCA and PSA lectins in the positive structural components.

DISCUSSION

In the present study, the cytochemical properties of differential sugar chain expression in different structural components of lingual tonsil were elucidated using 16 lectins from four major groups (N-acetylglucosamine, N-acetylgalactosamine, galactose, and mannose/ glucose groups). To date, only a few studies have been performed on the tonsils in which different cell types have been characterized by lectins. The specific varying patterns of lectins in the cell population of different layers of epithelium, connective tissue, blood vessels, and lymphatic, glandular and muscular tissues not only reflect their active localization and particular function but also neglect the possibility of passive binding. In the present study, the different lectins of a particular group showed almost similar expression for a particular tissue or group of cells; however, when comparing the positive affinities of different groups of lectins for a particular structure, minute differences were also found. It was a difficult task to compare the present results of buffalo tongue tonsils with those of other domestic animals because of the lack of relevant literature.

A strong binding pattern of WGA, s-WGA, LEL, DSL, and STL lectins in the reticular epithelium and stratum spinosum cells of stratified squamous epithelium indirectly reflected that the N-acetylglucosamine group was the major glycoconjugate in these cells. The differences in the staining patterns of the two epithelia were probably attributed to different environments and functions, as reported in the horse [10]; however, no differences were observed in the human tonsils [4]. The reticular epithelium has been described as an antigen collection site facilitated by the contents of the crypt [12, 13] and contributing to the effector functions of the mucosal immune system [12]. The absence of these sugars in the other layers suggests that it is a structural requirement and may be responsible only for spinosum cell growth and maintenance. In contrast, glycoconjugates with an N-acetylgalactosamine end group were less required in these cells, as reflected by their weak affinity for SBA, DBA, and RCA, with the exception of the VVL lectin. Similarly, these lectins showed a weaker affinity for reticular epithelium compared with the N-acetylglucosamine group. The glycoconjugates with galactose, mannose, and glucose groups were not detected in the different layers except for the lectins jacalin and Con A. Their presence in different cells reflects that glucose and mannose are other sugars required for proliferation and growth. However, the reason for the absence of PNA, ECL, PSA, and LCA lectins could not be explained; a similar report was available [14]. It was interesting to note that, with the exception of α -linked mannose and glucose, the cell architecture of the stratum granulosum did not have any of the carbohydrate components examined in the present study. Binding to galactose-specific components, particularly the lectin jacalin and the lectin Con A, which is also mannose/glucose specific, was a characteristic feature of the basal cells, indicating the presence of multiple residues in the terminal sugars. The most superficial layers were labeled by different lectins with different intensities, as previously reported [14, 15]. The presence of a variety of lectins on the surface epithelial cells indicates the presence of specific cell receptors formed by the interaction with the feeding particles or by degenerative changes, thus providing a better opportunity for the microorganisms to attach and penetrate [3].

All lectins with terminal N-acetylglucosamine, galactose, mannose, and glucose groups exhibited a differential affinity for subepithelial connective tissue, in which collagen fibers predominate. The response was weaker for the lectin s-WGA than for the other lectins of the N-acetylglucosamine group, suggesting that the isolation of the lectin may have blocked surface epitopes on the surface of the connective tissue. The lectins in the N-acetylgalactosamine group showed comparatively weaker positivity, with the exception of the lectin RCA, which showed a strong response. This could be due to the presence of an additional terminal sugar, lactose. A maximum positive reaction was observed for the lectins PNA, Con A, and PSA. The qualitative differences in positive response could be due to variations in terminal sugars or the presence of additional polysaccharide units. This could also be related to the presence of different types of collagen fibers. The remaining connective tissue of the propria submucosa showed comparatively lower affinity, with the exception of the tissue surrounding the glandular acini, where moderate activity was observed.

Lymphoid tissue components also showed variable positivity for the different lectins. The lectin WGA

labeled only a few lymphoid cells of the parafollicular and interfollicular areas, indicating the likely affinity of the lectin for macrophages, etc. The lectins STL and LEL were only weakly bound to the lymphoid cells, except for a few cells that might be macrophages of the interfollicular areas. The lectins s-WGA and DSL showed no affinity for any type of lymphoid cells. Similarly, all lectins of the N-acetylgalactosamine group were negative for all components of lymphoid tissue. Terminal galactose lectins were present in all lymphoid tissue cell populations except those of the germinal center in the present study. The presence of a reaction with ECL lectin in the lymphoid cells of the germinal center indicates the presence of galactose receptors on the B cells. No positive response was detected for the lectins PNA and SBA, as previously reported [15], but PNA and SBA showed an affinity for the germinal centers of hyperplastic human tonsils [14,16,17] and for histiocytes and macrophages [18]. The lectins Con A, PSA, and LCA of the mannose and glucose group showed binding affinity to the membrane and cytoplasm of some lymphoid cells of the germinal center, parafollicular and interfollicular areas, as well as to lymphocytes and macrophages. The structures that showed a very strong positive response might be macrophages since cytoplasmic binding of Con A was considered a selective marker for macrophage histiocytes in humans, suggesting binding to Golgi bodies, lysosomes, and other related structures [15, 16]. The differences in lectin expression in human and buffalo lymphoid cells could be due to species differences. A few lymphoid cells were detected in subepithelial connective tissue and reticular epithelium. Vascular cell adhesion molecule-I and intercellular adhesion molecule-I, which are responsible for mediating lymphocyte infiltration, were expressed by the reticular epithelium [4]. The present findings explain the dominance of glucose and mannose receptors on the surface of germinal center B cells, followed by galactose and glucosamine receptors on the surface of lymphoid cells of the inter- and parafollicular areas. However, the different cell types can be further identified by immunohistochemical studies. The lectins that bind to the lymphoid cell receptors can be used as cell markers to study lympho-proliferative disorders, as described previously [16].

Blood vessel endothelium had the highest concentration of N-acetylglucosamine in the present study, followed by N-acetylgalactosamine, galactose, glucose, and mannose. The extent of variation was probably due to the presence of different terminal sugars. A strong response was observed in the sections incubated with N-acetylglucosamine sugars. This was attributed to the binding of positively charged lectins to negatively charged sialyl residues in the glycocalyx of the mammalian endothelium [19]. The comparatively lower intensity for the neutral lectin s-WGA could be due to less binding with sialyl residues. This also confirmed the negatively charged surface coatings of the vascular endothelium in laboratory animals [20]. Although the lectins of the N-acetylgalactosamine group have similar terminal sugars, RCA and VVL showed stronger affinity for the vascular endothelium compared with the other two lectins of the same group. The lectins RCA-I and BS -I, which possess non-reducing β -galactosyl residues, have been described as lectins specific for vascular endothelium in humans and domestic animals [21, 22]. The endothelium of blood vessels also possessed galactose receptors, which showed a moderate response. The lectin PNA also showed no binding sites in the endothelium of blood vessels, as previously reported [14]; however, a positive response was detected after pre-incubation with neuraminidase [21]. The lectins PSA and LCA showed a negative to weak response in the endothelium, which is consistent with previous observations [20] and suggests a lower amount of mannose and glucose components in the endothelium.

The mucous glandular tissue exhibited different reactions for different groups of lectins and also for the different lectins of the same group, indicating the specificity of sugar moieties. The lectins of the N-acetylglucosamine group presented varying positive affinity for the secretions of the glandular acini. The lectins WGA and DSL showed a weak reaction to mucous secretions, whereas s-WGA was strongly positive, indicating that the secretions have a predominance of N-acetylneuraminic acid (sialic acid). The lectins LEL and STL had moderate positivity for the secretions. The lectins of this group were localized to only a few glandular acini, and the reaction was not observed in the majority of the mucous acini. In addition, their patterns of localization were different in the lobes of the glandular acini. The mucous secretions had comparatively less concentration of N-acetylgalactosamine, as evidenced by their binding affinity. The activity for lectin GS1B4 was moderate to strong, localized to only a few acinar cells of the glandular acini. The lectins with galactose specificity, except jacalin, did not show an affinity for the mucous secretions. However, a strong reaction was observed

towards the periphery of the acini in the region of myoepithelial cells, fine blood capillaries, and connective tissue containing collagen fibers. The lectin jacalin showed a strong homogeneous positive reaction throughout the glandular acini without delineation of the myoepithelial cells. Similarly, the secretions of only a few glandular acini and myoepithelial cells were moderately positive for the lectins of mannose and glucose groups. The lectins of a particular group are bound similarly to a specific type of cell that may be used as a specific tool to carry out further investigations regarding their functional status, especially in disease conditions. However, the minor variations in staining affinities may be attributed to the differentiation of cell types or maturation of the glycosylation chain attached to the secretory protein [23]. Lectins having the property to identify molecules characteristically can be utilized as modern tools in histochemistry and immunohistochemistry for structural and functional analysis of cells, tissues, and microorganisms [24]. The limitation of the present study was the lack of facilities for quantification of the positivity of the lectins in different structures.

CONCLUSIONS

The present study characterized the specific lectin binding affinities to the structural components of the lingual tonsil in buffaloes. The cells of stratum spinosum showed a maximum affinity for the lectins of the N-acetylglucosamine group amongst all the groups of the lectins, followed by glucose/mannose groups of lectins during the present study. The stratum granulosum and stratum corneum did not exhibit positivity for any of the sugar residues except α -D-mannose, and glucose, as demonstrated by Con A in the former stratum. Most superficially placed keratinized cell layers were varyingly positive for the majority of the lectins. The reticular epithelium could be demonstrated weakly by almost 14 lectins. The lectins of N-acetylglucosamine followed by galactosamine and galactose groups were choices of lectins to expedite the vascularity pattern of the endothelial cells of the blood vessels. The lymphoid cells of inter and parafollicular areas possess galactose receptors, whereas the B cells can be marked by the lectins of the glucose/mannose group. The glandular secretions positive for mucopolysaccharides have a predominance of terminal sugars D-GlcNAc.

ACKNOWLEDGEMENTS

The author sincerely acknowledges the award of the research grant received vide No. HSCST/R&D/ 2018/2101 Dt. 01.08.2018 by Haryana State Council for Science and Technology, Panchkula, India.

CONFLICT OF INTEREST

The author does not have any conflict of interest.

FUNDING STATEMENT

The author did not receive any funding from a private agency to carry out this research work.

REFERENCES

[1] Girgiri IA, Pawan K. Histological and histochemical studies on the lingual tonsil of the buffalo (Bubalus bubalis). J Buffalo Sci 2019; 8: 68-76. https://doi.org/10.6000/1927-520X.2019.08.03.3

[2] Girgiri IA, Pawan K. Scanning and transmission electron-microscopic studies on the lingual tonsil of the buffalo (Bubalus bubalis). J Buffalo Sci 2020; 9: 5-12. https://doi.org/10.6000/1927-520X.2020.09.02

[3] Sharon N, Lis H. Lectins as cell recognition molecules. Sci 1989; 246: 227-34. https://doi.org/10.1126/science.2552581

[4] Clark MA, Hirst BH, Jepson MA. Lectin-mediated mucosal delivery of drugs and microparticles. Adv Drug Deliv Rev 2000; 43: 207-23. https://doi.org/10.1016/S0169-409X(00)00070-3

[5] Kuru N, Cinar K, Demirbag E, Ilgun R. Some lectin binding properties of the tongue of the mole rat, *Nannospalax xanthodon*. Biotech Histochem 2017; 92: 445-49. https://doi.org/10.1080/10520295.2017.1341057

[6] Bies C, Lehr CM, Woodley JF. Lectin-mediated drug targeting: history and applications. Adv Drug Deliv Rev 2004; 56: 425-35. https://doi.org/10.1016/j.addr.2003.10.030

[7] Haltner E, Easson JH, Lehr CM. Lectins and bacterial invasion factors for controlling endo-and transcytosis of bioadhesive drug carrier systems. Eur J Pharma Biopharmaceutics 1997; 44: 3-13. https://doi.org/10.1016/S0939-6411(97)00096-9

[8] Russell-Jones GJ, Veitch H, Arthur L. Lectin-mediated transport of nanoparticles across Caco-2 and OK cells. International Journal of Pharmaceutics. 1999; 190: 165-74. https://doi.org/10.1016/S0378-5173(99)00254-9

[9] Smart, JD. Lectin-mediated drug delivery in the oral cavity. Adv Drug Delivery Rev 2004; 56: 481-89. https://doi.org/10.1016/j.addr.2003.10.016

[10] Kumar P, Timoney JF. Histology and ultrastructure of the equine lingual tonsil. I. Crypt epithelium and associated structures. Anat Histol Embryol 2005; 34: 27-33. https://doi.org/10.1111/j.1439-0264.2004.00560.x

[11] Kumar P, Timoney JF. Histology, immunohistochemistry, and ultrastructure of the tonsil of the soft palate of the horse. Anat Histol Embryol 2006; 35; 1-6. https://doi.org/10.1111/j.1439-0264.2005.00622.x

[12] Perry M, Whyte A. Immunology of the tonsils. Immunol Today 1998; 19: 414-21. https://doi.org/10.1016/S0167-5699(98)01307-3

[13] Gebert A, Pabst R. M cells at locations outside the gut. Immunol 1999; 11: 165-70. https://doi.org/10.1006/smim.1999.0172

[14] Wirbel R, Möller P, Schwechheimer K. Lectin-binding spectra in the hyperplastic human tonsil. Effect of formalin fixation

and paraffin embedding on lectin affinity of tissue components. Histochemistry 1984; 81: 551-60. https://doi.org/10.1007/BF00489534

[15] Hsu SM, Ree HJ. Histochemical studies on lectin binding in reactive lymphoid tissues. J Histochem Cytochem 1983; 31: 538-46. https://doi.org/10.1177/31.4.6827084

[16] Strauchen JA. Lectin receptors as markers of lymphoid cells. I. Demonstration in tissue section by peroxidase technique. American J Pathol 1984; 116: 297-304.

[17] Reisner Y, Biniaminov M, Rosenthal E, Sharon N, Ramot B. Interaction of peanut agglutinin with normal human lymphocytes and with leukemic cells. Proc National Acad Sci 1979; 76: 447-51. https://doi.org/10.1073/pnas.76.1.447

[18] Howard DR, Batsakis JG. Peanut agglutinin: a new marker for tissue histiocytes. Am J Clin Pathol 1982; 77: 401-8. https://doi.org/10.1093/ajcp/77.4.401

[19] Monsigny M, Roche AC, Sene C, Maget-Donica R, Delmotte F. Sugar lectin interactions: How does wheat germ agglutinin bind sialoglycoconjugates? Eur J Biochem 1980; 104: 147-53. https://doi.org/10.1111/j.1432-1033.1980.tb04410.x

[20] Skutelsky E, Rudich Z, Danon D. Surface charge properties of the luminal front of blood vessel walls: An electron microscopical analysis. Thromb Res 1975; 7: 623-34. https://doi.org/10.1016/0049-3848(75)90108-5

[21] Alroy J, Goyal V, Skuteisky E. Lectin histochemistry of mammalian endothelium. Histochemistry 1987; 86: 603-07. https://doi.org/10.1007/BF00489554

[22] Gerhart DZ, Zionis MS, Drewes LR. Light and electron microscopic localization of D-galactosyl residues in capillary endothelial cells of the canine cerebral cortex. J Histochem Cytochem 1986; 34: 641-48. https://doi.org/10.1177/34.5.3701030

[23] Nakajima T, Shiratori K, Ogawa K, Tanioka Y, Taniguchi K. Lectin-binding patterns in the olfactory epithelium and vomeronasal organ of the common marmoset. J Vet Med Sci 1998; 60: 1005-11. https://doi.org/10.1292/jvms.60.1005

[24] Santos AFS, da Silva MDC, Napoleão TH, Paiva PMG, Correia MTS and Coelho LCBB. Lectins: Function, structure, biological properties, and potential applications. Cur Topics Peptide Protein Res 2014; 15: 41-62.

Received on 17-03-2023 Accepted on 08-05-2023 Published on 16-05-2023

https://doi.org/10.6000/1927-520X.2023.12.08