Full Length Research Paper

Histochemical demonstration of mucosubstances in the mouse gastrointestinal tract treated with *Origanum hypericifolium* O. Schwartz and P.H. Davis extract

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Glycoconjugate-containing mucous in the gastrointestinal tract has lubricant and cytoprotective effects. This study was performed to demonstrate the intensity of mucous in the stomach, small and large intestine tissues of female BALB/c mice injected intraperitoneally with 0.2 ml of the O*riganum hypericifolium* extract for six weeks. The staining procedures employed were Alcian Blue (AB) at pH 1 and 2.5, periodic acid-Schiff (PAS) and lectins including *Galanthus nivalis* agglutinin (GNA), *Arachis hypogaea* (PNA, Peanut Agglutinin) and *Maackia amurensis* leucoagglutinin (MAL). The mucous glands in the large intestine of the experimental groups were found to show stronger positive reaction for AB at pH 2.5 and PAS when compared with the controls. In lectin histochemistry, the mucous glands which reacted with lectins exhibited less density for PNA in the stomach and for MAL and PNA in the small intestine in the experimental groups than the controls. In the large intestine of the experimental groups, less staining pattern of GNA was observed on the surface epithelium of villi when compared with the controls. The results suggest that *O. hypericifolium* extract increases acidic and neutral mucosubstances in the large intestine and also changes some glycan structures in the gastrointestinal tract.

Key words: Mucosubstances, Gastrointestinal tract, Glycoconjugates, *Origanum hypericifolium*.

INTRODUCTION

The specialized secretory cells of gastrointestinal tract produce the protective and lubricant substance, mucous. Mucous are composed mainly of large glycoproteins called mucins and inorganic salts. The gel-like properties of mucous are due to its complex glycoproteins. Glycoproteins serve as antioxidative properties in the gastrointestinal tract. Since structural alterations in mucous can lead to some gastrointestinal diseases, the studies on the change of mucosubstances along the gastrointestinal tract in pathologic problems have a great

importance. Hereby, many investigations have shown an interaction between the changes in mucosubstances and gastrointestinal disorders. For instance, it has been reported that the reduction in mucosubstances in the gastric secretion on exposure to stress, plays an important role in the development of lesions in stomach (Debnath et al., 1980). Pearson and Brownlee (2010) also noted that the normal functionality and biochemistry of the mucous barrier appears to be lost in diseases of the colorectal mucosa. In peptic ulcer, the deficiency of gel forming polymeric mucins of the antral adherent mucous barrier (Allen et al., 1988) and the reduction of acid mucosubstances in goblet cells (Morrissey et al., 1983) have also been reported.

Carbohydrates both on the outer surface of cellular and secreted macromolecules, mediate many events in cell-cell and cell-matrix interactions leading to the develop-ment and functions of organisms. Consequently, several

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Table 1. Lectin characteristics.

Abbreviation	Lectin source	Carbohydrate binding specificity
MAL	*Maackia amurensis*	NeuAc α2-3Gal
GNA	*Galanthus nivalis*	α 1-3 and α 1-6 linked high mannose structures
PNA	*Arachis hypogaea*	Galβ1-3GalNAc

human diseases are characterized by changes in carbohydrates, so they are highly considered in therapeutic investigations (Varki et al., 1999). It has been hypothesized that the alterations in mucous glycoproteins in gastrointestinal diseases play an important role in the pathogenesis of the diseases such as ulcerative colitis, Crohn's disease (Rhodes et al., 1988) and colon cancer (Aoki et al., 1993).

Therefore, the detection of histochemical alterations in sugar compositions of mucous in the gastrointestinal diseases have received great attention (Kuhlmann et al., 1983; Ishikawa, 1994; Lueth et al., 2005). Lectins have been used as useful probes to detect glycoconjugates, thereby elucidating functional and pathologic problems in various cells of gastric epithelia related to the change of glycoconjugates. Along with conventional histochemical methods, lectin histochemistry provides additional data, improving analysis on the distribution of specific glycosubstances in the mucosa.

Many medicinal plants exhibiting antioxidant and cytoprotective activities have been studied with regard to gastrointestinal disorders. The ulcer protective effect of methanolic extract of fresh roots of *Asparagus racemosus* has been demonstrated. It has been reported that the plant significantly increased mucous secretion as the mucosal defensive factors (Sairam et al., 2003). Similarly, gastroprotective effects of *Pongamia pinnata* (Prabha et al., 2009), *Azadirachta indica* (Dorababu et al., 2006) and *Cissus quadrangularis* (Jainu et al., 2006) have been reported. In addition, the increase in mucin secretion and glutathione levels and the decrease in lipid peroxidation in gastric mucosa of diabetic rats treated with *Eugenia jambolana* plant extract were reported. The plant extract is therefore thought to be a choice for treating gastric ulcers co-occurring with diabetes (Chaturvedi et al., 2009). *Origanum hypericifolium* belongs to Lamiaceae family and is broadly known as thyme. It is an endemic species in Denizli Region of Western Turkey (Davis, 1982) and it's extract contains mainly p-simenep-cymene, γ-terpinene, thymol and carvacrol (Celik et al., 2010). It has been shown that monoterpens like carvacrol have antioxidant and antitumor activities.

The aim of this study was to demonstrate the possible changes in glycoconjugates of the mucosubstances of the gastrointestinal tract in mice treated with *O. hypericifolium* ex*t*ract, so as to investigate the relationship between mucous secretion and the plant extract, applying a variety of histochemical techniques.

MATERIALS AND METHODS

Plant extraction

To study the effects of *O. hypericifolium* extract on mucosubstances in the gastrointestinal tract of mice, the aerial parts of the plant were collected during the flowering period between the months of June and July, 2009 from Sandras Mountain (elevation 1860 m), Beyağaç-Denizli, Turkey. The voucher specimens were deposited at the herbarium of Biology Department, Faculty of Science and Art, Pamukkale University (herbarium no. AÇE 2545). The samples were air-dried and stored in a polyethylene bag. At the time of the experiment, 10 g of fragmented aerial parts of the plant were put into the boiling distilled water. The mixture was left to brew for 10 min. Then, the plant parts were filtered from the mixture and the remaining brew was stored at +4°C.

Experiments were performed at Experimental Research Center, Faculty of Medicine, Pamukkale University, Denizli, Turkey. A total of 16 BALB/c female mice ranging between 6 to 9 weeks in age were used for the study. They were allocated into two groups with eight mice in each group. Group I: Control animals, water was administrated for 6 weeks; Group II: Test animals, the plant extract (0.2 ml) was injected intraperitoneally three times a week for 6 weeks. Animals were sacrified under anesthesia two days after the last extract injection.

Histochemistry and lectin histochemistry

For histochemistry, biopsies of stomach (from fundus), small intestine (from ileum) and large intestine (from colon) were fixed in Sainte-Marie's fixative (Sainte-Marie, 1962). After routine tissue processing, samples were embedded in paraffin. 6 µm thick sections from paraffin blocks were cut by using a microtome (Leica, RM 2145) and stained as follows: periodic-acid-Schiff (PAS) (McManus, 1948) for neutral mucosubstances; alcian blue (AB) (Mowry, 1956; Lev and Spicer, 1964) for acid (pH 2.5) and sulphated (pH 1) mucosubstances counterstained with neutral red. For lectin histochemistry, the specimens were immersed in the optimal cutting temperature (OCT) compound blocks in liquid nitrogen for cryostat technique. They were stored at -86°C until use. 6 µm thick sections were cut by using a cryotome (Leica, CM 1510 S). The sections were then mounted on 0.01% (w/v) poly-L-lysine-coated microscope slides, which were allowed to air-dry, and stored at -20°C for one day. The lectins used were digoxigenin-labelled lectins [*Galanthus nivalis* agglutinin (GNA), peanut agglutinin (PNA), *Maackia amurensis* leucoagglutinin (MAL or MAAI)] (DIG glycan differentiation kit, Roche Diagnostics, Germany) (Table 1). Negative controls, in which they were run by omitting the lectins, were included in the analysis. Olympus BX50 light microscope and olympus DP2-BSW microscope digital camera system were used
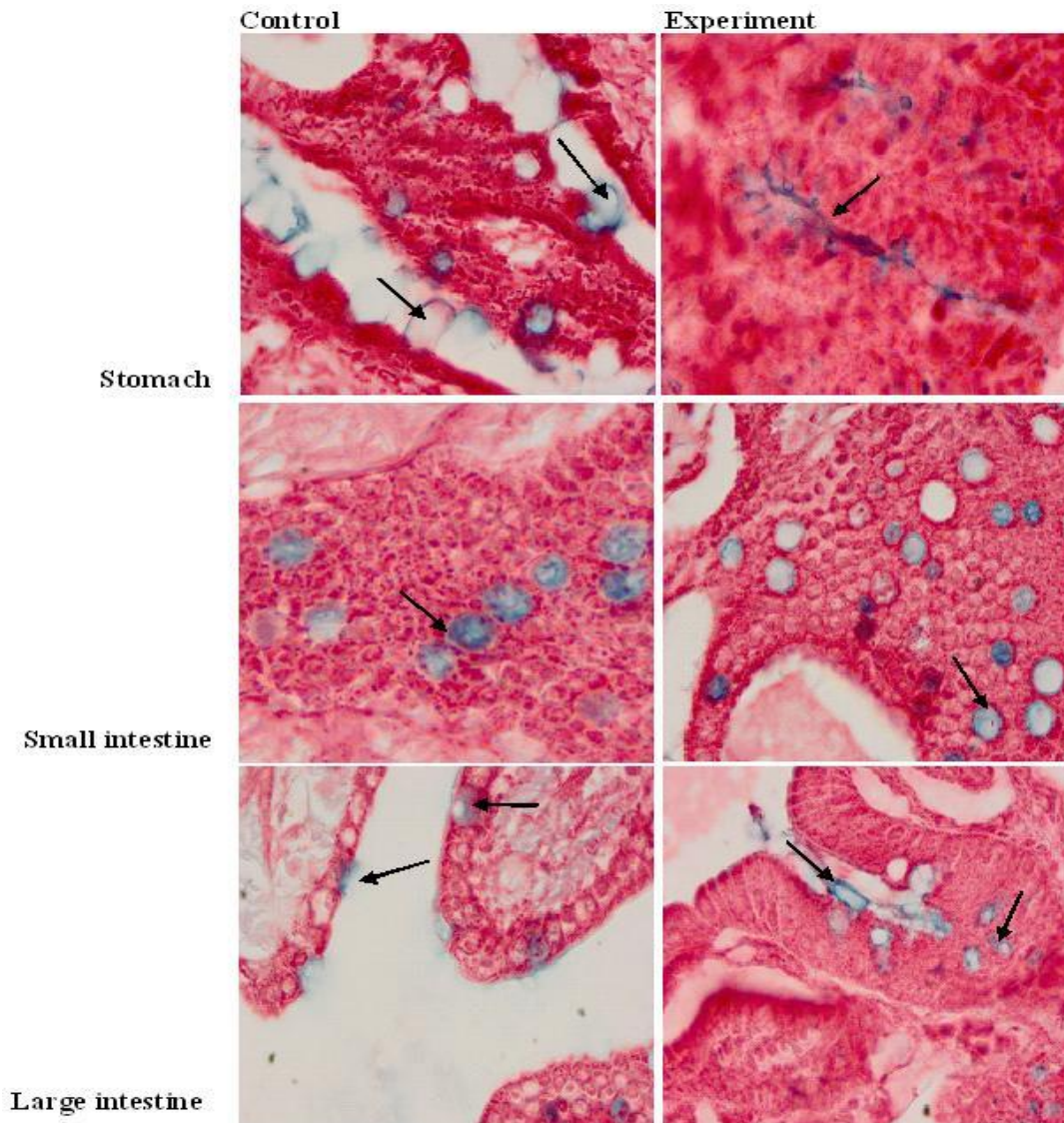
Figure 1. Photomicrographs of histological cross sections of the stomach, small and large intestine, weakly stained with AB at pH 1. In all the micrographs, the mucosubstances are indicated by arrows (x 1000).

for photography.

RESULTS

Histochemical results

Sulfo mucins and acidic mucins

Sections from the stomach, stained with AB at pH 1 and 2.5 indicated the presence of sulfated and acidic glycoconjugates. Positive reactions was observed mainly in the epithelium, the gastric pits in the mucosa and the glands in the lamina propria for both groups. In the small intestine of both groups, the intensity of AB reactions at pH 1 and 2.5 were same in the acid and sulphated

mucins, respectively. However, in the large intestine, the 2.5 in the experimental groups than the controls. The density of AB-positive reaction at pH 1 was weaker than at pH 2.5 in all groups (Figures 1 and 2).

Neutral mucins

A PAS-positive reaction was found predominantly in the apical surface of the goblet cells in the gastrointestinal tract. In the stomach and small intestine, there were no significant intensity differences in the neutral mucin staining with PAS. On the other hand, in the large intestine, PAS-positive mucous material was more densely stained in the experimental groups than in the
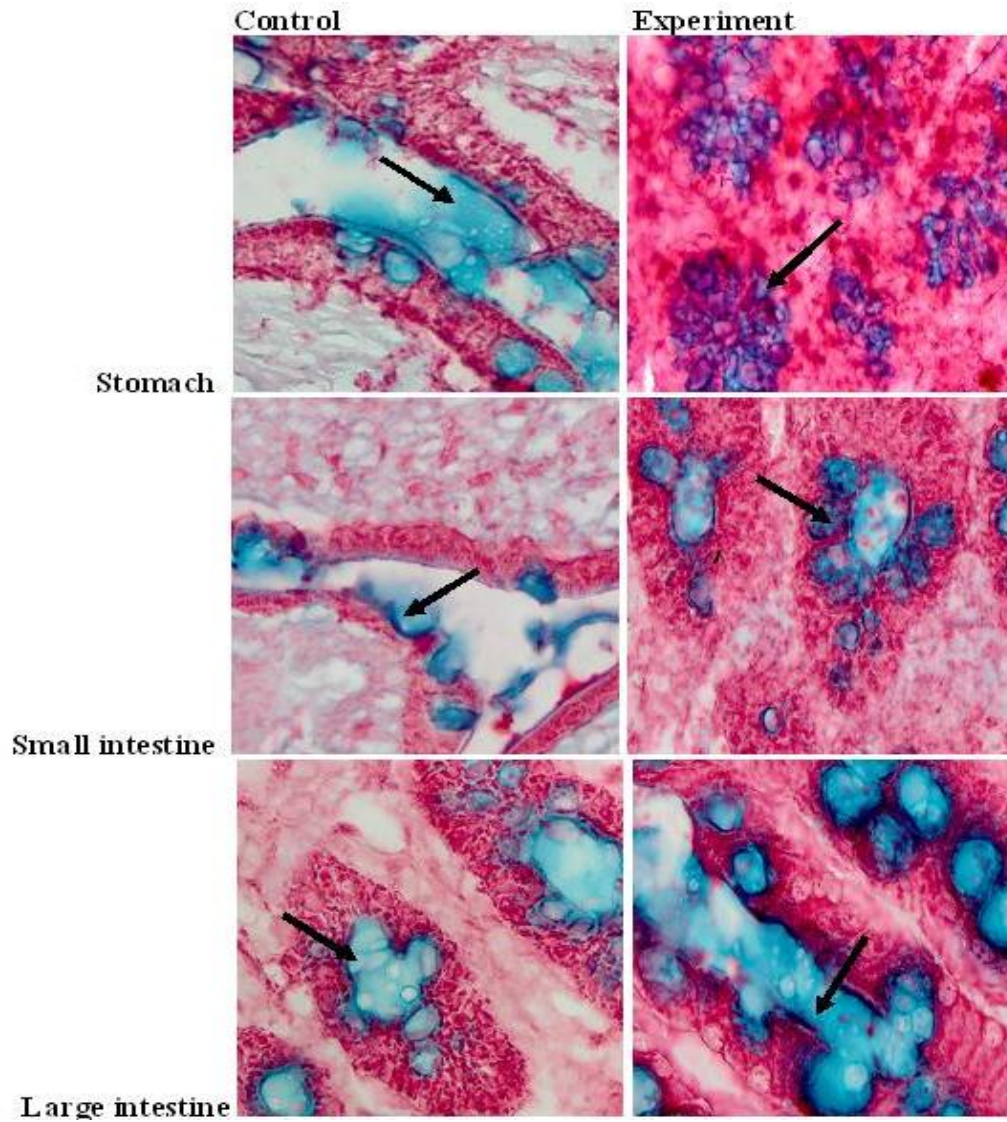
Figure 2. Photomicrographs of glandular portions of the stomach, small and large intestine, strongly stained mucosubstances with AB at pH 2.5 (arrows). Note the intense stain of the mucosubstances in the large intestine in the experimental groups than the controls (x1000).

controls (Figure 3). Table 2 shows the reactivity mucins was slightly more densely stained by AB at pH grades of the neutral, acidic and sulfomucins in the stomach, small and large intestines by the above mention histochemical staining methods.

Lectin histochemical results

The lectin stainings in the mucosa of stomach, small and large intestine are shown in Table 3 for both the control and experimental groups.

In general, the staining patterns with MAL on the surface epithelium of the villi, with GNA to the mucous cells were similar for both groups in the stomach. On the

other hand, PNA reactivity decreased in glands in the experimental groups when compared with the controls (Figure 4).

In the small intestine, more dense reaction with MAL was observed on the surface epithelium of villi in the controls than in the experimental groups.

However, GNA staining intensity was similar for both groups. On the other hand, weaker PNA reactivity was found in mucous glands in the experimental groups when compared with the controls (Figure 5). In the large intestine, generally, similar MAL and PNA reactivities were observed on the surface epithelium of villi for both groups, whereas less reactivity of GNA was observed in the experimental groups when compared with the controls (Figure 6).
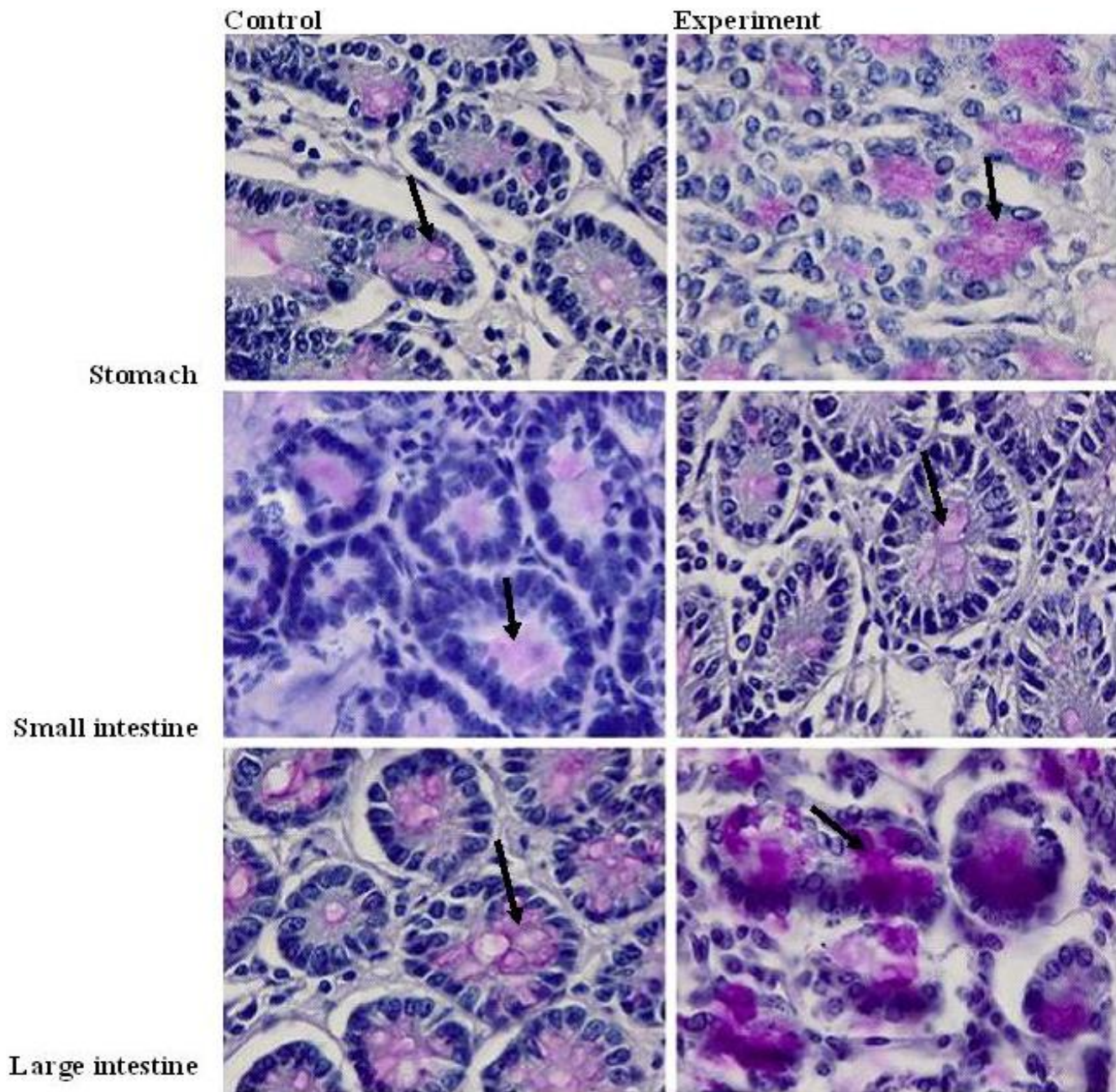
Figure 3. PAS positive material (arrows) in apical surfaces of the gland cells in the stomach, small and large intestines. Note the densely stained mucosubstances in the experimental groups of the large intestine (x1000).

DISCUSSION

The present work presents histochemical investigations of the glycoconjugates in mucosal epithelium of the stomach, small and large intestines of mice treated with O. *hypericifolium* extract. The mucous glands in large intestine of the experimental group revealed positive reactivities with AB pH 2.5 for acidic and PAS for neutral mucosubstances. These results indicate that plant extract can increase mucous secretion containing acidic and neutral carbohydrates from glands in the large intestine. Presumeably, it may increase the production of glycoproteins of mucosubstances. From this hypothesis, it can be pronounced that further investigations are needed for analysis of the effects of the plant extract on the mucin synthesis. On the other hand, we could not find any staining differences in density in any group at pH 1 for sulfated mucosubstances. In addition, the staining pattern at pH 1 was weaker than the staining pattern at pH 2.5. Since acidic mucosubstances have the lubricating and

Table 2. Histochemical reaction of neutral, acidic and sulfated acidic mucosubstances of the stomach, small and large intestines in the controls and experimental groups.

Epithelial Mmucosubstance	Histochemical staining intensity					
	AB pH 1		AB pH 2.5		PAS	
	Control	Experiment	Control	Experiment	Control	Experiment
Stomach	+++	+++	++++	++++	++	++
Small intestine	+++	+++	++++	++++	++	++
Large intestine	++	++	++++	+++++	++	+++

Intensity of reaction: ± (pale reaction) to +++++ (strongest reaction).

Table 3. Intensity of lectin binding in the stomach, small and large intestines from the controls and experimental groups.

Epithelial Mucosubstance	Lectins with carbohydrate specificity					
	MAL-sialic acid α(2→3)galactose		GNA-terminal mannose		PNA-galactoseβ(1→3)N-acetylgalactosamine	
	Control	Experiment	Control	Experiment	Control	Experiment
Stomach	++	++	+++	+++	+++±	+++
Small intestine	++++	+++	+++±	+++±	++++	++
Large intestine	++++	++++	++++	+++	++++	++++

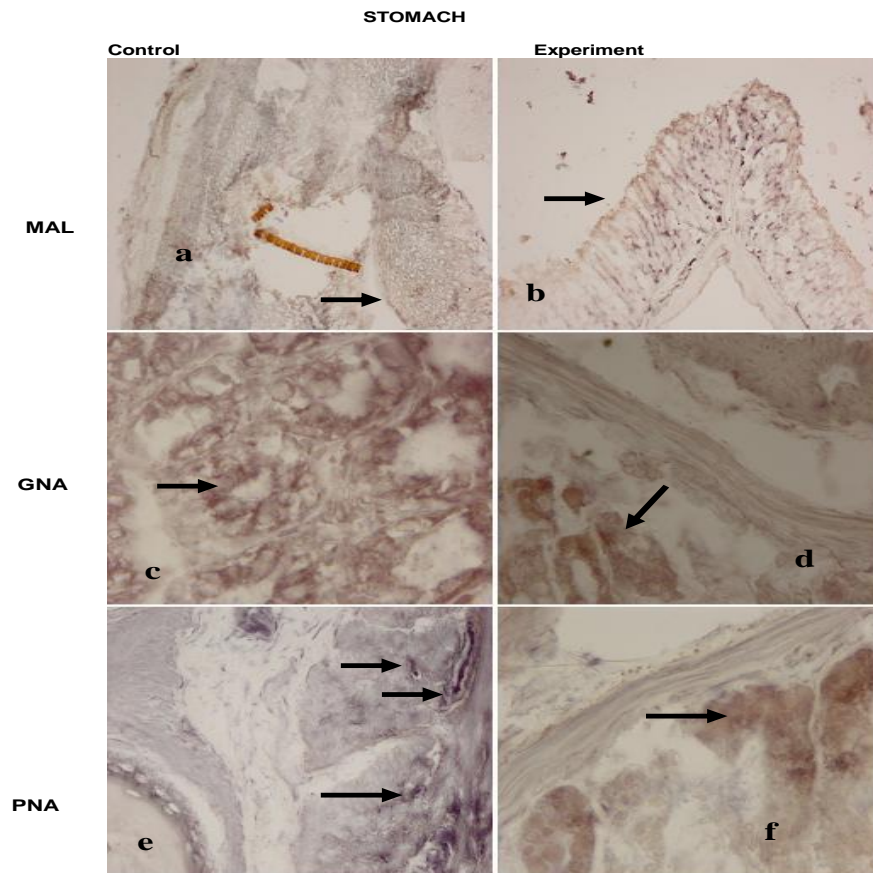Intensity of reaction: ± (pale reaction) to ++++ (strongest reaction).

Figure 4. The lectin binding patterns (arrows) of the stomach. x100 for a, b; x1000 for c, d, e and f).
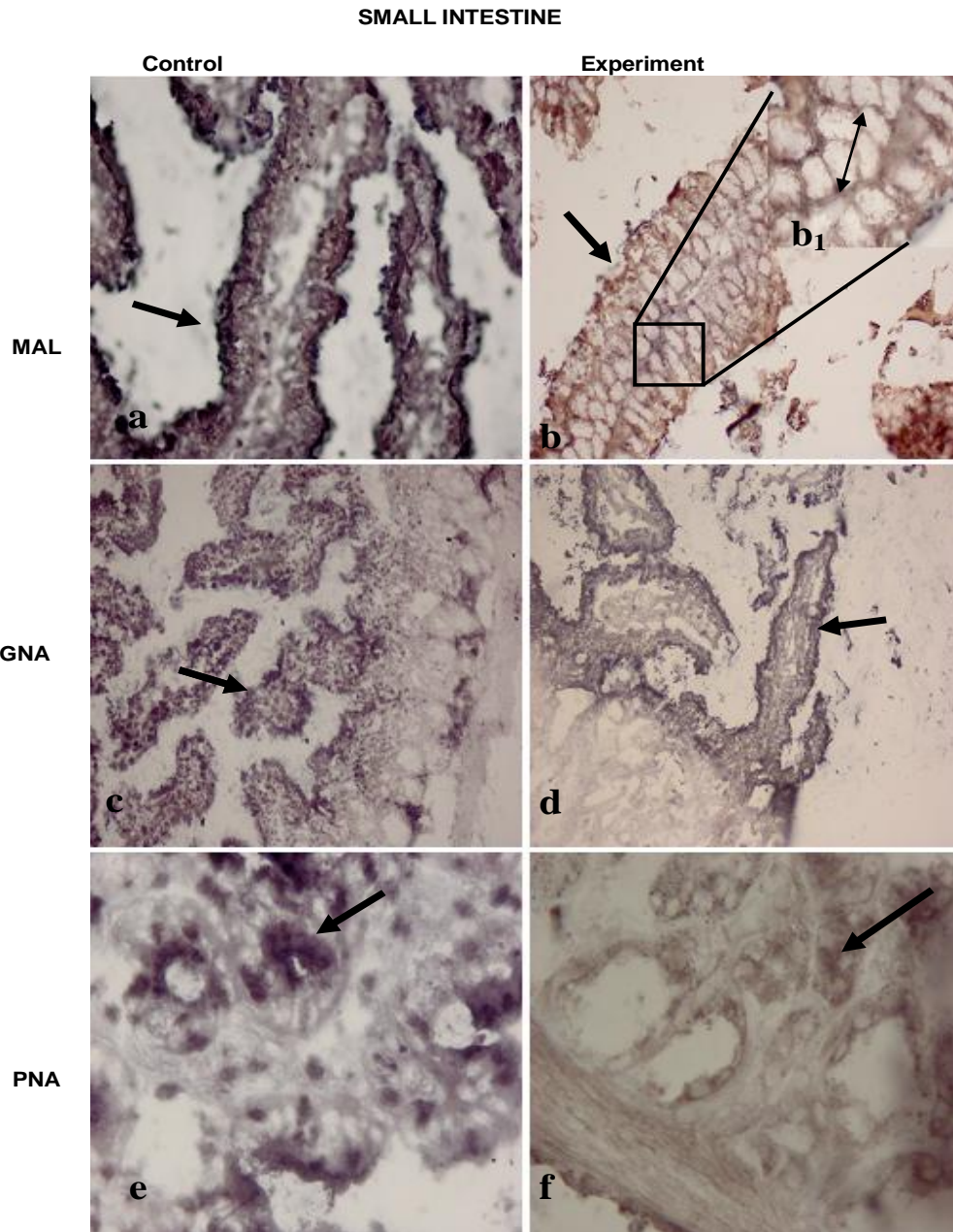
SMALL INTESTINE

Figure 5. The lectin binding patterns (arrows) of the small intestine. The MAL+ reaction (double arrow) in the cell membrane of mucous cells (b_1). 400x for a, b_1; x100 for b; x200 for c, d; x1000 for e and f).

protective functions (Taib, 1984; Mousa et al., 1956), and/or cell surface mucosal barriers (McGuckin et al., 2011), the increase of the secretion of acidic mucosubstances in the large intestine in the experimental groups may be important particularly for potential pathogens.

Observed lectin staining patterns showed that in the stomach for PNA, in the small intestine for MAL and PNA and in the large intestine for GNA, had weaker stainings in the experimental groups as compared to their controls.

In other words, the results obtained from analysis of the three lectins used here indicated that the structures of Galβ1-3GalNAc in stomach and small intestine, NeuAc α2-3Gal in small intestine and α 1-3 and α 1-6 linked high mannose in the large intestine decreased in the experimental groups. Thus, the plant extract has a slight decreasing effect in terms of some glycan structures in the gastrointestinal tract.

However, an increase of acidic mucosubstances in the large intestine in the experimental group was detected.
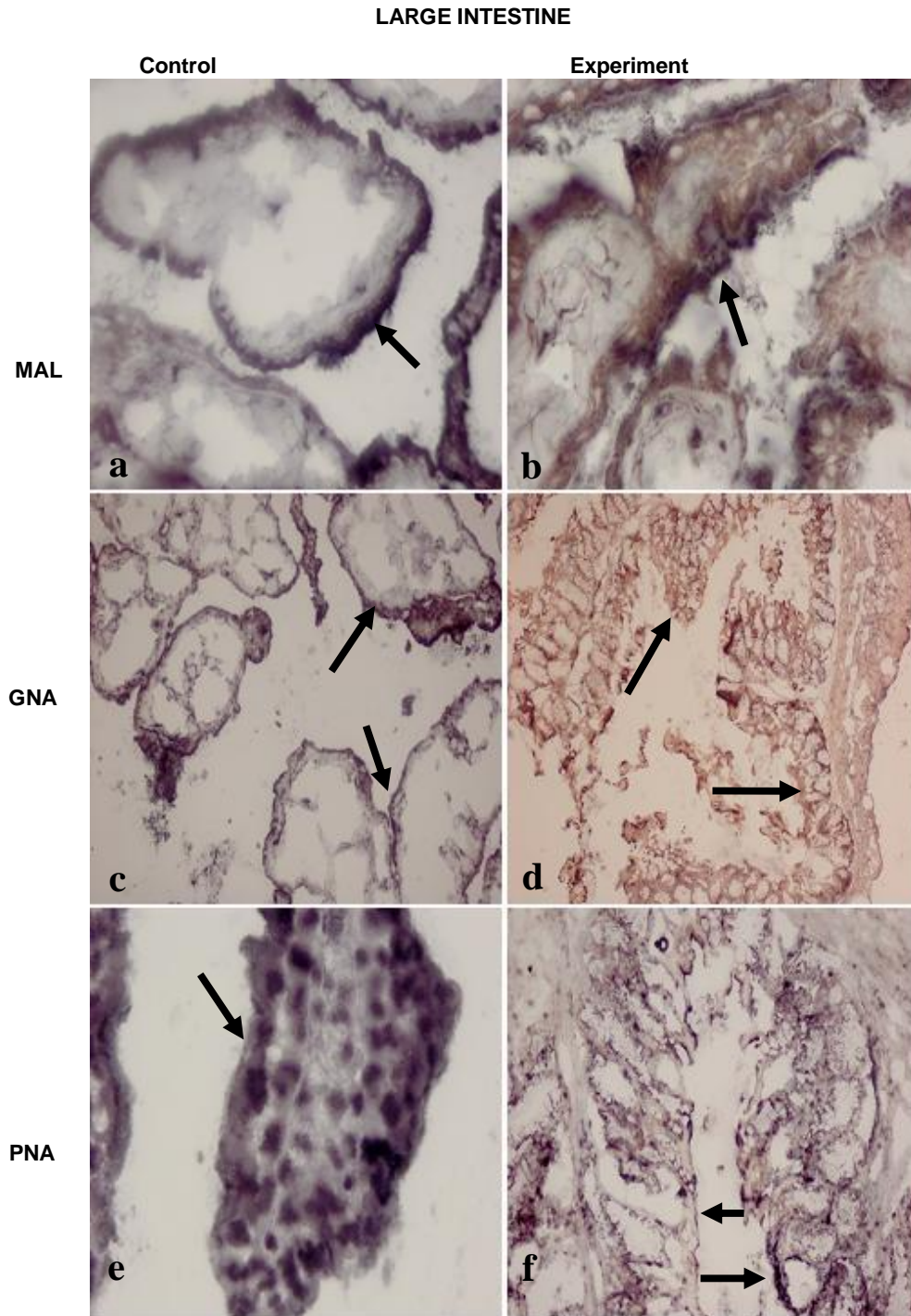
LARGE INTESTINE

Figure 6. The lectin binding patterns (arrows) of the large intestine. GNA showed weak staining in the experimental groups than the controls. (x400 for a, b; x200 for c, d, e and f).

Therefore, these findings have necessity for further analysis for research on the correlation of muco-substances/O. *hypericifolium* extract in the gastro-intestinal tract.

The use of a number of drugs for the treatment of peptic ulcer disease causes relapses, side effects and drug interactions (Dharmanl and Pallt, 2006). Therefore, it can be proposed that investigations on herbal sources for gastrointestinal disorders may have a great importance for the development of new protective drugs. There have been many researches on this topic (Chen et al., 2005; Al Mofleh et al., 2008; Koo and Cho 2004; Suba et al., 2004;

Nguelefack et al., 2005). Several studies related to the pharmacological effects of some *Origanum* species on the gastrointestinal disorders have also been reported (Dundar et al., 2008; Al-Howiriny et al., 2009). Celik et al. (2010) detected the major compounds found in volatiles of *O. hypericifolium* as p-cymene (14%), carvacrol (74%) and γ-terpinene (35%) which have antioxidant capacity. Pathogens disrupt and avoid mucosal barriers in some gastrointestinal disorders. Therefore, the effects of the extract of *O. hypericifolium* on mucosubstances may be important in the mechanism of the interactions between pathogens and mucins.

In this study, histochemical analyses revealed that *O. hypericifolium* extract causes slight changes in the intensity of acid mucosubstances in the large intestine and in some glycan moieties in the gastrointestinal tract. However, further investigations are needed in order to understand the molecular basis of the effects of *O. hypericifolium* extract on mucosubstances of gastrointestinal tract.

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