Effects of Aluminium Chloride Exposure on the Histology of the Small intestine of Wistar Rats

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Abstract

The small intestine is the largest component of the digestive tract and the major site of digestion and absorption. Aluminium compounds are used in pharmaceuticals and in water treatment processes. The purpose of this experiment was to evaluate the possible effects that aluminium chloride exposure could have on the histology of the small intestine of wistar rats. The wistar rats were divided into five groups as follows: Group I was the control that received distil water only while Groups II-V were given various concentrations of aluminium chloride via oral intubation for period of eight weeks. The wistar rats were humanely sacrificed, the small intestine removed, fixed, processed and stained in haematoxylin and eosin (H&E).The slides were viewed under the light microscope fitted to a digital camera. Based on our observations, we therefore conclude that aluminium chloride exposure had negative and deleterious effects on the histology of small intestine of wistar rats as eminent in mucosa degeneration.

Keywords: Aluminium Chloride, histology, small intestine,wistar Rats.

1. Introduction

The small intestine (or small bowel) is the part of the gastrointestinal tract following the stomach and followed by the large intestine, and is where much of the digestion and absorption of food takes place. In invertebrates such as worms, the terms "gastrointestinal tract" and "large intestine" are often used to describe the entire intestine. The primary function of the small intestine is the absorption of nutrients and minerals found in food, as well as, completing the digestion of food delivered into it by the stomach (Sherwood, 2006; Solomon. et al., 2002; Arthur, 1974).

The small intestine is the largest component of the digestive tract and the major site of digestion and absorption. In addition to receiving chyme from the stomach, the initial segment of the small intestine, the duodenum, receives bile from the gall bladder and digestive enzymes from the pancreas. The pancreatic enzymes are produced in an inactive form and only become active in the lumen of the duodenum. The small intestine is divided into three parts, the duodenum (25 cm), the jejunum (2.5 m) and the ileum (3.5 m).

The duodenum is the first part of the small intestine, followed by the jejunum and ileum (in that order); it is also the widest and shortest part. The duodenum is a C-shaped or horseshoe-shaped structure that lies in the upper abdomen near the midline (Gray and Lewis, 2000; Agur, et al., 1999; Grant, et al., 1989). In fish, the divisions of the small intestine are not as clear, and the terms anterior intestine or proximal intestine may be used instead of duodenum (Latunde-Dada, et al., 2002; Guillaume, et al., 2001). In mammals the duodenum may be the principal site for iron absorption (Duodenal Anatomy). The duodenum precedes the jejunum and ileum and is the shortest part of the small intestine, where most chemical digestion takes place.

In humans, the duodenum is a hollow jointed tube about 25-38cm (10-15 inches) long connecting the stomach to the jejunum. It begins with the duodenal bulb and ends at the ligament of Treitz. (van Gijn and Gijselhart, 2011). The mucosa of the small intestine is highly modified. The luminal surface is completely covered by a number of finger-like or leaf-like projections called villi, 0.5-1.5 mm in length. The core of a villus is an extension of the lamina propria, and its surface is covered by a simple columnar epithelium. Opening onto the luminal surface at the bases of the villi are simple tubular structures called intestinal glands or crypts of Lieberkuhn. The crypts extend downward toward the muscularis mucosae. The simple columnar epithelium lining them is continuous with that covering the villi.

The predominant cell type of the epithelium is the enterocyte or absorptive cell. Each enterocyte has about 3000 microvilli at its luminal surface, which appear in the light microscope as the fuzzy striated border on the surface of the villi. Microvilli are cylindrical protrusions, about 1 micrometer tall, of the cell membrane enclosing a core of filaments, mostly actin filaments. The actin filaments attach to the plasma membrane at the tip of the microvillus and end in the terminal web near the base of the microvillus. The terminal web consists of actin microfilaments and myosin, and is attached to the zonula adherens of the junctional complex binding epithelial cells to one another near their apical ends.

The villi and microvilli, together with folds in the submucosa called plicae circulares (below), increase the absorptive surface of the small intestine about 600 times.

The epithelium of the small intestine consists of the following cell types:

- **Enterocytes** or **absorptive cells**. These are tall columnar cells with microvilli and a basal nucleus, specialized for the transport of substances. They are bound to one another and other cell types by junctional complexes (zonula occludens or tight junction, zonula adherens, and macula adherens). Amino acids and monosaccharides are absorbed by active transport, monoglycerides and fatty acids cross the microvilli membranes passively. Absorbed substances enter either the fenestrated capillaries in the lamina propria just below the epithelium, or the lymphatic lacteal (most lipids and lipoprotein particles). Enterocytes have a lifespan of about 5-6 days.
- **Goblet cells**. These mucus-secreting cells are the second most abundant epithelial cell. They are found interspersed among the other cell types. Their mucous is a very large glycoprotein that accumulates at the apical end of the cell, rendering it wide. The slender base of the cell holds the nucleus and organelles. Goblet cells usually appear pale or empty due to the loss of their contents upon preparation. The abundance of goblet cells increases from the duodenum to the terminal ileum. Their lifespan is also 5-6 days.
- **Paneth cells**. Paneth cells are found only in the bases of the crypts of Lieberkuhn. These cells have an oval basal nucleus and large, refractile acidophilic granules at their apical end. The granules contain the antibacterial enzyme lysozyme, other glycoproteins, an arginine-rich protein and zinc, an essential trace metal for a number of enzymes. Paneth cells also phagocytize some bacteria and protozoa. They may have a role in regulating intestinal flora. They have a lifespan of about four weeks.
- **Enteroendocrine cells**. In the intestine, they are most often found in the lower part of the crypts but can occur at all levels of the epithelium. Their most abundant products here are cholecystokinin or CCK, which stimulates pancreatic enzyme secretion and gall bladder contraction, secretin, which stimulates pancreatic and biliary bicarbonate secretion, and gastric inhibitory peptide or GIP, which inhibits gastric acid secretion. As in the stomach, these cells are not easily seen without special preparations.
- **Microfold cells**. These cells are epithelial cells that overlie Peyer's patches and other large lymphatic aggregations. They are relatively flat and their surface is thrown into folds, rather than microvilli. They endocytose antigens and transport them to the underlying lymphoid cells where immune responses to foreign antigens can be initiated.
- **Undifferentiated cells**. These stem cells are found only at the base of the crypts and give rise to all the other cell types. A cell destined to be a goblet cell or enterocyte undergoes about 2 additional divisions after leaving the pool of stem cells, and migrates from the crypt to the villus. It will be shed at the tip of the villus.

Lymphocytes are sometimes seen in the intestinal epithelium. They are thought to be sampling antigens in the epithelial intercellular spaces.

It is believed that they process the antigens before returning to lymphatic nodules in the lamina propria and undergoing blastic transformation, leading to antibody secretion by the newly differentiated plasma cells. Within the lamina propria core of each villus is a lymphatic capillary called a lacteal, as well as numerous capillaries. The lacteal is accompanied by smooth muscle fibres arising from the muscularis mucosae. The smooth muscle in the villus allows it to contract intermittantly, expelling the contents of the lacteal into the lymphatic network surrounding the muscularis mucosae. The lamina propria is very cellular, with numerous lymphocytes, plasma cells, macrophages and eosinophils. Lymphatic nodules arising in the lamina propria may extend into the submucosa. The muscularis mucosae may be partially or totally disrupted by the nodules.

The submucosa consists of dense connective tissue. Adipose cells may be present. Both the duodenum and the jejunum are characterized by modifications of the submucosa. (So is the ileum, although its modification, Peyer's patches, arises from the lamina propria.) The duodenum is distinguished by the presence of Brunner's glands, which occupy most of the submucosa**.** In some areas these glands may penetrate the muscularis mucosae to enter the lamina propria. Brunner's glands are branched tubuloalveolar glands that produce a clear, viscous, alkaline (pH 8.1-9.3) fluid, containing neutral and alkaline glycoproteins and bicarbonate ions. The secretion of Brunner's glands protects the proximal small intestine by neutralizing the acidic chyme from the stomach. It brings the pH of the intestinal contents close to the optimum for the pancreatic digestive enzymes delivered to the stomach. Jejunum is characterized by the plicae which consist of a core of submucosa and the overlying mucosa. They have a semilunar, circular or spiral form and extend about one-half to two-thirds around the circumference of the lumen. Although they may be present in the duodenum and ileum, they are not as large and are not a significant feature in those regions (Sherwood, 2006; Solomon. et al., 2002).

Aluminium has the potential to be neurotoxic in human and animals. It presents in many manufactured foods and medicines and is also added to drinking water for purification purposes (Newairy *et al*., 2009). Aluminium is widely used in antacid drugs, as well as in food additives and tooth paste (Abbasali *et al*., 2005). Environmental pollution with different aluminium containing compounds, especially those in industrial waste expose people to higher than normal levels of Aluminium (Kloppel *et al.*, 1997).

Aluminium is also thought to be a causal agent in some cases of encephalopathy and osteomalacia observed in patients with chronic renal failure caused by long-term hemodialysis (Tahara, 2004). Aluminium toxicity in humans has been implicated in many neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, and parkinsonism-dementia (Roberts, 1986; Garruto and Brown, 1994; Solomon et al., 2001).The mechanism of aluminium-inducted neurotoxicity and identification of effective treatment for such impairments is, therefore, an important public and occupational health priority for industrial and developing nations. Aluminium contributes to a variety of cognitive impairments in mice, rabbits, and rat pups (Muller et al., 1990; Yokel, 1985, Bilkei-Gorzo, 1993; Mari et al., 2001). Epidemiological studies have indicated a link between Aluminium in drinking water and AD and a variety of human and animal studies have implicated learning and memory deficits after Aluminium exposure (Buraimoh et al., 2011a; Exley, 2005; Schmidt et al., 2001; Yokel, 2000). The aim of this study was to evaluate the possible effects that aluminium chloride exposure could have on the histology of small intestine of wistar rats.

2. Materials and Methods

This experiment was conducted in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Samaru, Zaria, Nigeria. The rules and regulations governing animal handling were observed.

2.1. Experimental animals

Twenty healthy looking wistar rats were used for this study. The wistar rats were kept in steel cages in the Department of Human Anatomy under good ventilation. They were properly fed with pelletized grower mash obtained in Samaru market of Zaria and there was supply of regular drinking water to the wistar rats. The wistar rats were kept in the Department for two weeks before the commencement of administration of aluminium chloride: this was done in order to allow the wistar rats acclimatized to the environment.

2.2. Experimental Design

The wistar rats were divided into five groups. Group I was the control that received distil water only. The remaining four groups were given various concentrations of aluminium chloride as follows:

Group II received 475mg Kg^{-1},
 Group III received 950mg kg^{-1},
Group IV received 1,425mg kg^{-1},
And Group V received 1,900mg kg^{-1} through oral intubation for a period of eight weeks.

2.3. Tissue processing and staining

The wistar rats were humanely sacrificed by anesthetizing them in a suffocating chamber using chloroform, after the end of eight weeks of administrations of various concentrations of aluminium chloride except the control group I that received distil water only. The abdominal region was dissected and the small intestine were removed, and immediately fixed in 10% formalin. After fixation, the small intestines were transferred into an automatic processor where they went through a process of dehydration in ascending grades of alcohol (ethanol) 70%, 80%, 95% and absolute alcohol for 2 changes each. The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotary microtome. The tissue sections were deparaffinised, hydrated and stained using the routine haematoxylin and eosin staining method (H&E). The stained sections were examined under the light microscope fitted to a digital camera and lap top for photomicrographs.
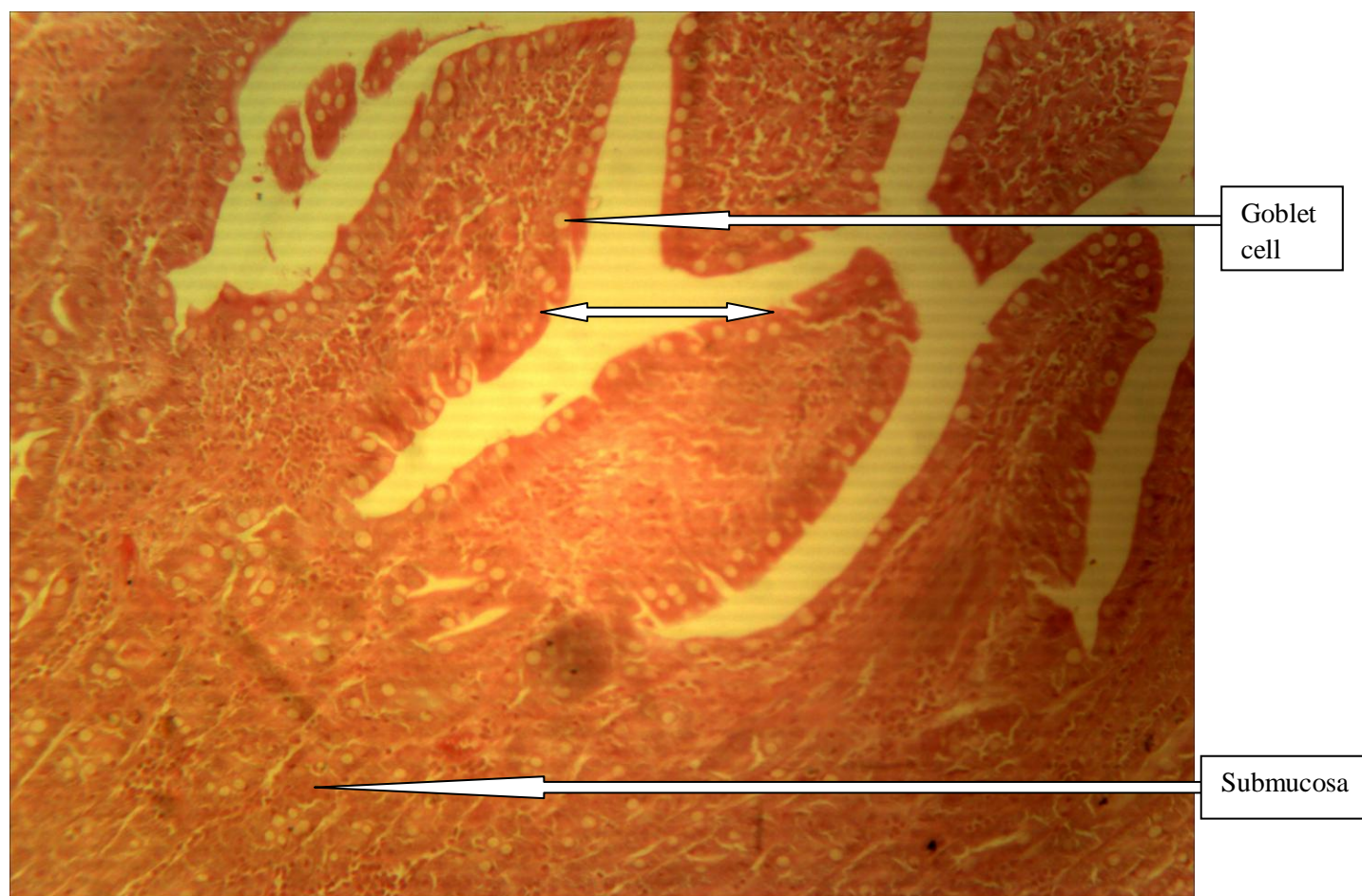
3. Results and Discussion

Plate 1. Photomicrograph of a section of the wall of the small intestine of the control group I showing normal submucosa(with brunner's glands), mucosa and tall cylindrical villi(double arrow) with goblet cells. X100 H&E.
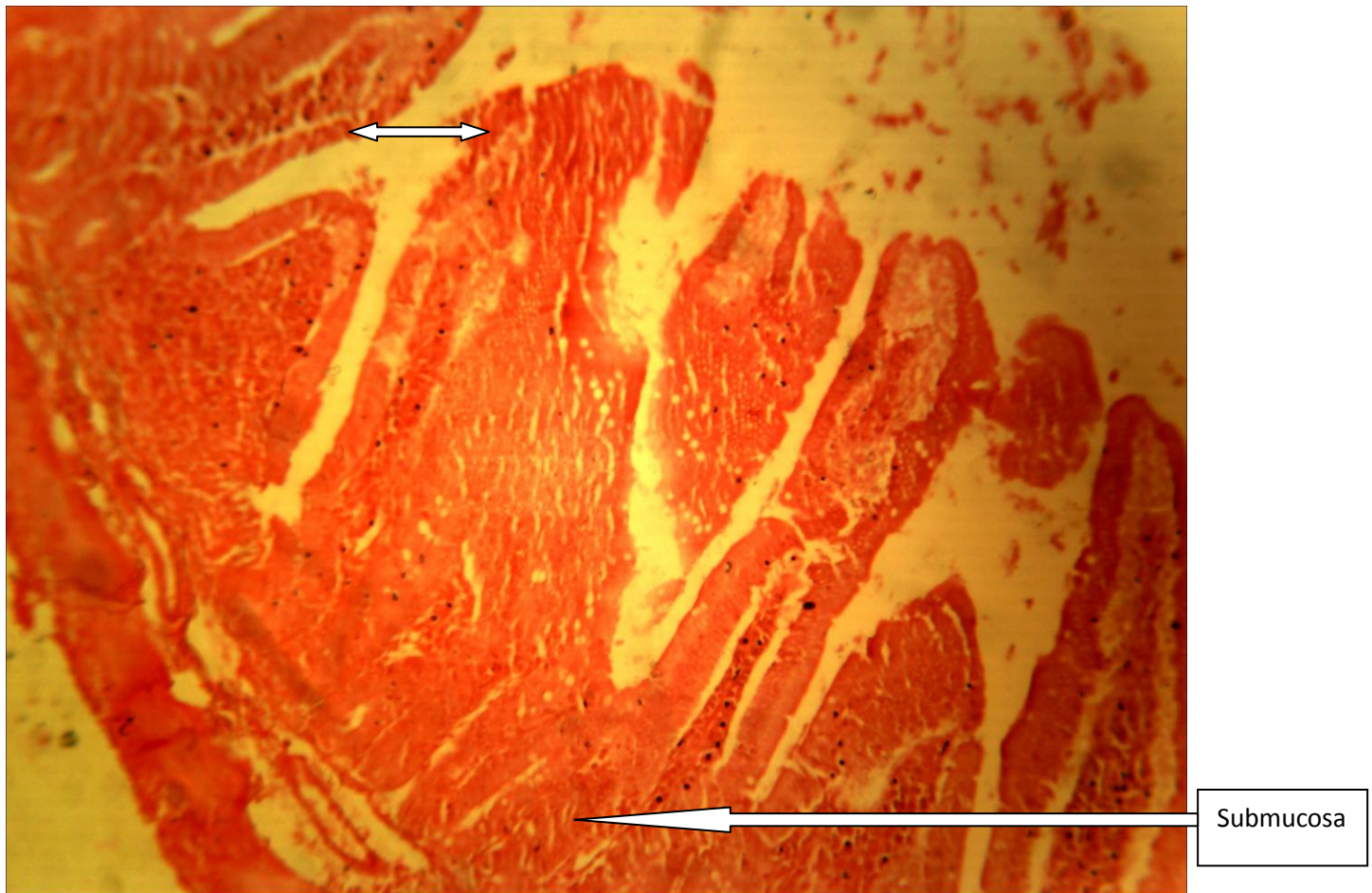
Plate 2 Photomicrograph of a section of the wall of the small intestine of the group II showing submucosa(with brunner's glands), mucosa degeneration and tall cylindrical villi(double arrow) with few goblet cell.X100 H&E.
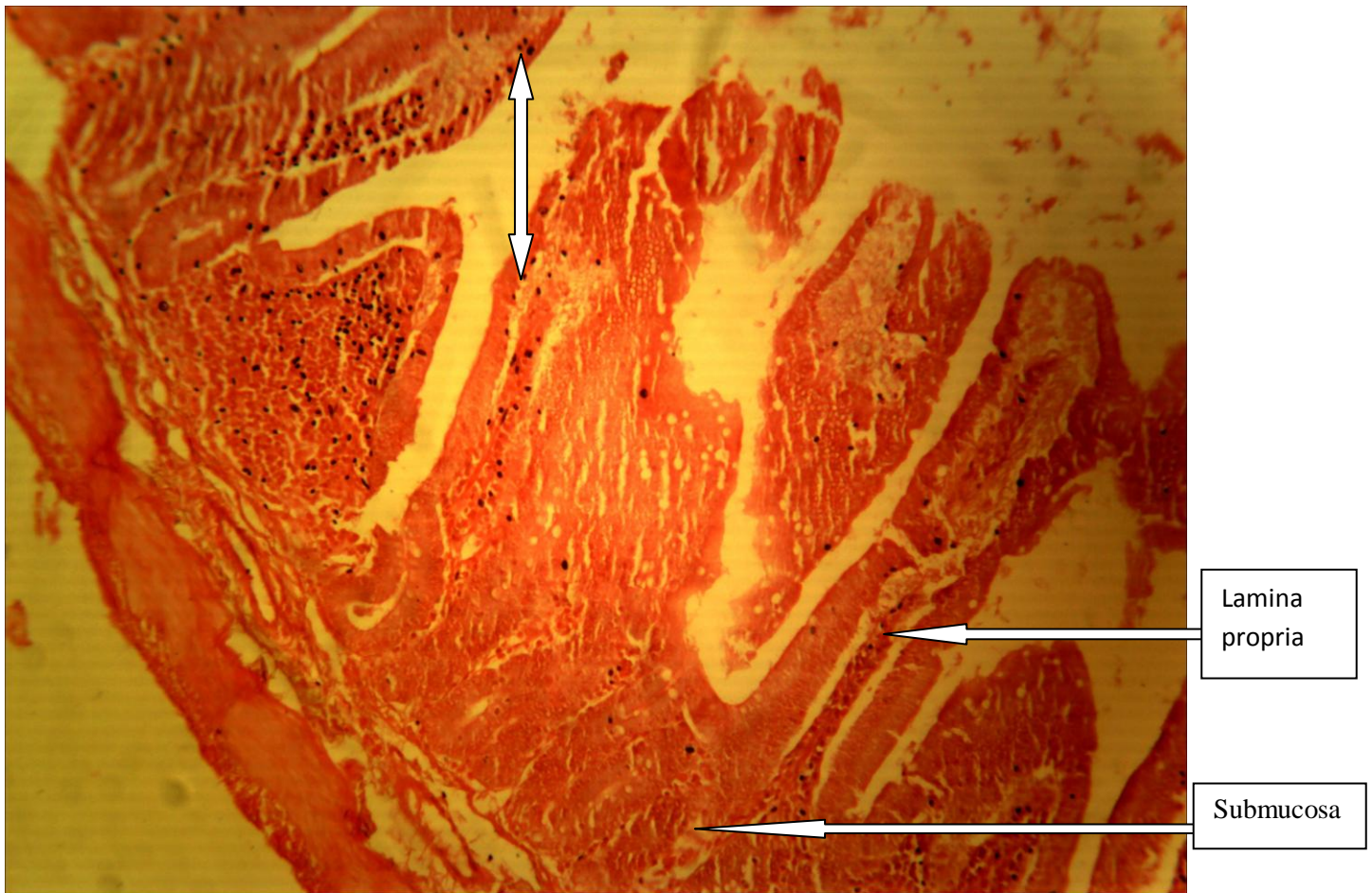
Plate 3. Photomicrograph of a section of the wall of the small intestine of the group III showing submucosa(with brunner's glands), mucosa degeneration and tall cylindrical villi(double arrow) with few goblet cell and proliferation of lymphocytes(black spots). X100 H&E.
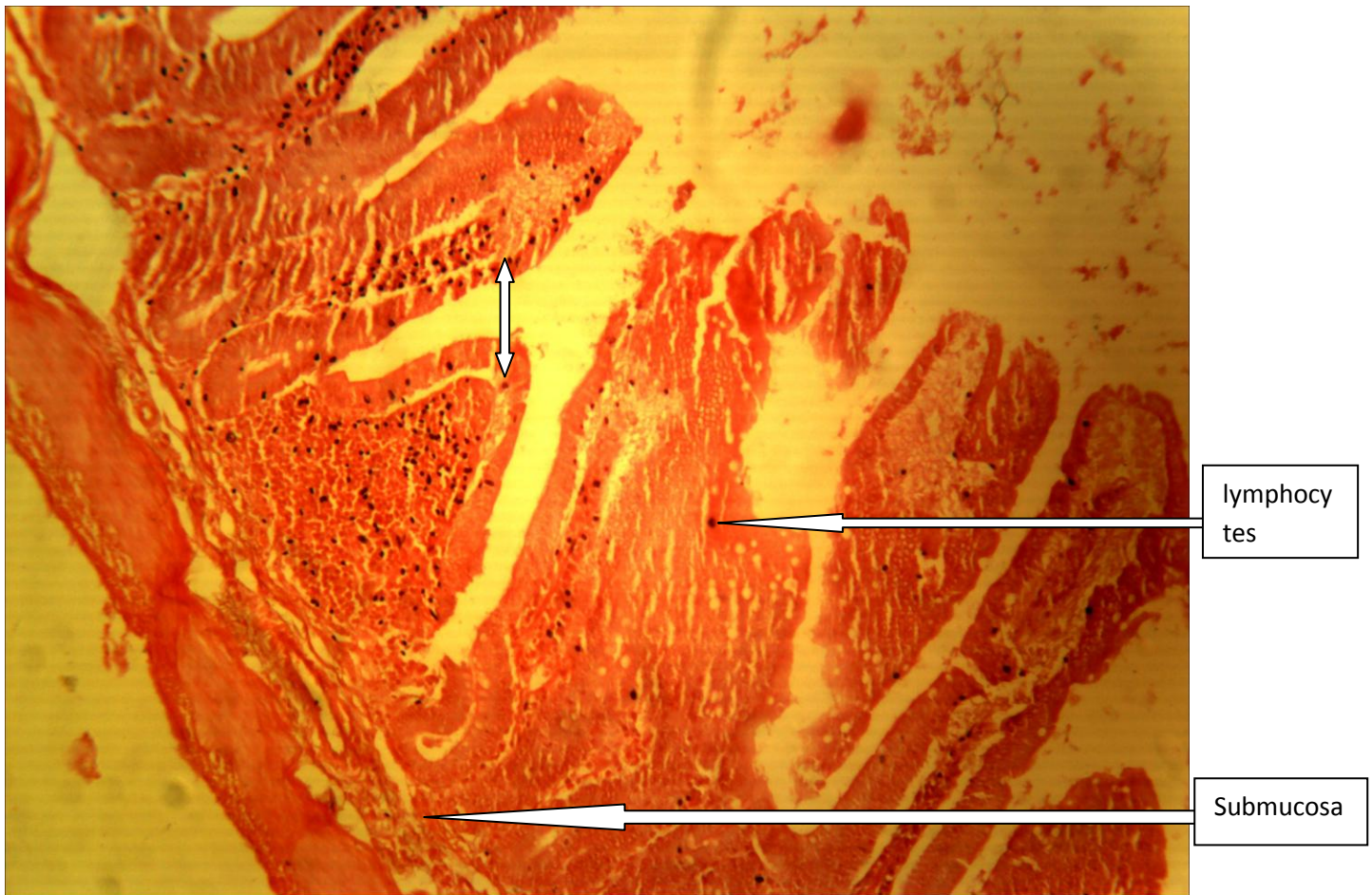
Plate 4. Photomicrograph of a section of the wall of the small intestine of the group IV showing submucosa(with brunner's glands), mucosa degeneration and tall cylindrical villi(double arrow) with few goblet cell and proliferation of lymphocytes. X100 H&E
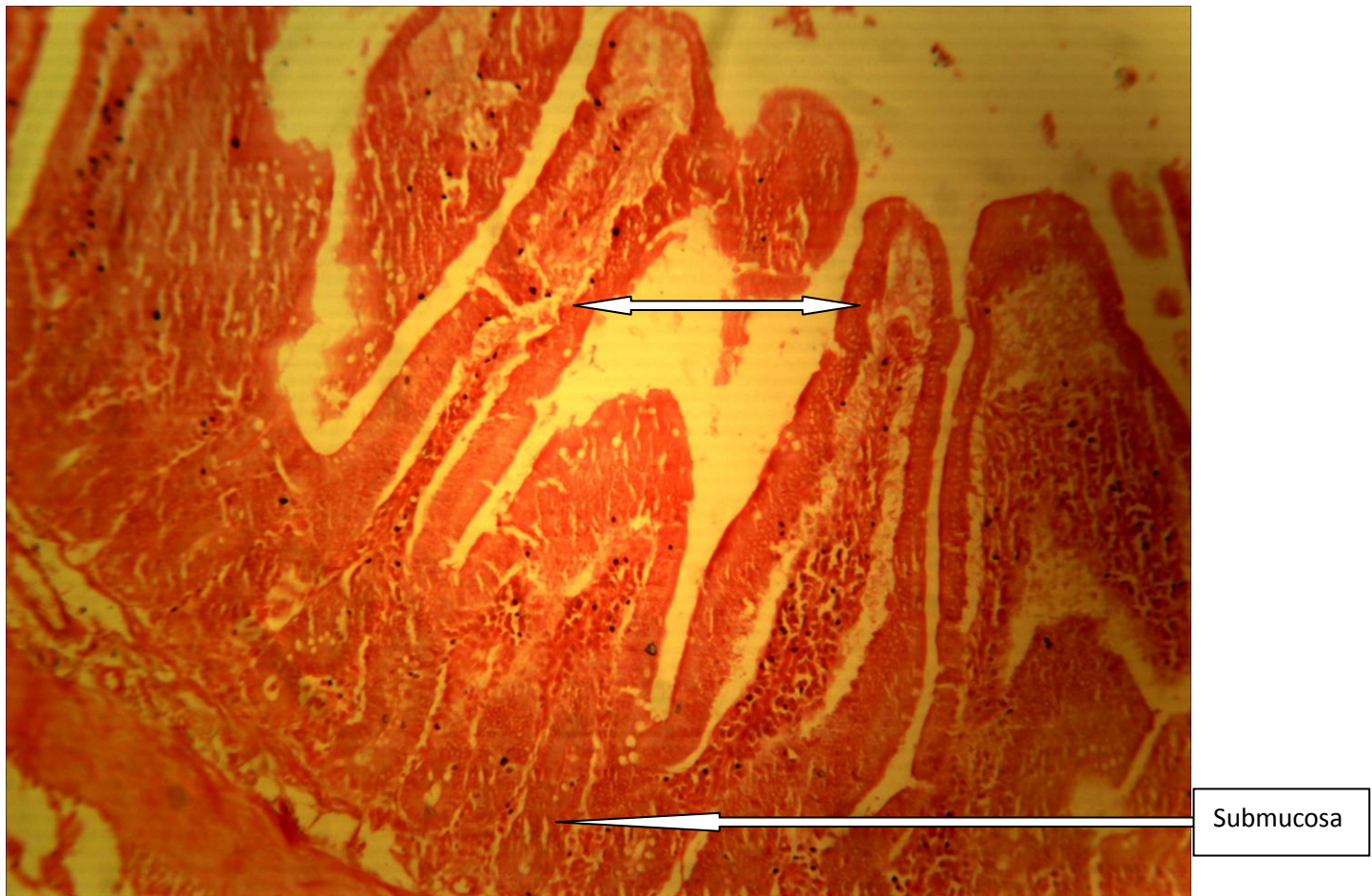
Plate 5. Photomicrograph of a section of the wall of the small intestine of the group V showing submucosa(with brunner's glands), mucosa degeneration and tall cylindrical villi(double arrow) with few goblet cell and proliferation of lymphocytes(black spots) X100 H&E.

According to Greger *et al*. (1985), aluminium in the food supply comes from natural sources including water, food additives and contamination by aluminium utensils and containers. Oral exposure to aluminium is from food, water and pharmaceutical products.

Role of Aluminium intoxication in different organs in neurodegenerative diseases has been recently emphasized (Somova, et al., 1997; Exley, 1999).

There is little indication that aluminium is acutely toxic by oral exposure despite its widespread occurrence in foods, drinking-water, and many antacid preparations (WHO, 1997). In 1988, a population of about 20,000 individuals in Camelford, England, was exposed for at least 5 days to unknown but increased levels of aluminium accidentally distributed to the population from a water supply facility using aluminium sulfate for treatment. Symptoms including nausea, vomiting, diarrhoea, mouth ulcers, skin ulcers, skin rashes, and arthritic pain were noted. It was concluded that the symptoms were mostly mild and short-lived. No lasting effects on health could be attributed to the known exposures from aluminium in the drinking-water (Clayton, 1989).

Aluminium chloride was implicated to have negative effects on behavioural endpoints of wistar rats(i.e. alters behaviour), have negative effects on anxiety-related behaviour of wistar rats as it increased the rate of anxiety in aluminium treated rats, had neurodegenerative effects on the histology of cerebral cortex of adult wistar rats especially at higher dose, was also said to have detrimental effects on the integrity of the testes of wistar rats , and also decrease the level of sperm count, but did not result into infertility.(Buraimoh, et al., 2011b; Buraimoh, et al., 2011c; Buraimoh, et al., 2012a; Buraimoh, et al., 2012b; Buraimoh, et al., 2012c).

In our present study,the Photomicrograph of the section of the wall of the small intestine of the control group I showed normal submucosa(with brunner's glands), normal mucosa and tall cylindrical villi(see Plate I). Photomicrographs of the section of the wall of the small intestine in group II, III, IV and V that received 475mg Kg^{-1},950mg kg^{-1}, 1,425mg kg^{-1} and 1,900mg kg^{-1} of aluminium chloride respectively showed few goblet cell, mucosa degeneration and proliferation of lymphocytes when compared with the the control group I (Plates I-V). This was in contrast with the observations of Buraimoh, et al., (2012d) on the epididymis where they stated that aluminium chloride exposure had no significant effects on the histology of the epididymis and may not be detrimental to the cyto-architecture of the epididymis of wistar rats; therefore, storage of sperm in the epididymis could be safe. But was in concord with another finding that stated that aluminium chloride exposure was detrimental to the histology of the kidney of wistar rats (Buraimoh and Ojo 2012).

In the present study, the mucosa degeneration observed in the wall of the sections of the small intestine in the aluminium treated groups was an indication of negative and deleterious effects it had on the treated groups when compared with the control (Plates II-V).

4. Conclusion

Based on our observations, we therefore conclude that aluminium chloride exposure had negative and deleterious effects on the wall of the small intestine of wistar rats as eminent in mucosa degeneration, few goblet cells and lymphocytes proliferation observed in the aluminium treated groups.

Acknowledgement

References

Abbasali, K.M., Zhila, T. & Farshad, N. (2005). Developmental Toxicity of aluminium from High Doses of AlCl3 in Mice. The Journal of Applied Research, 5: 575-579.

Agur, A.M.R., Lee, M.J. & Grant, J.C.B. (1999).*Grant's Atlas of Anatomy*. 10th Ed. London, UK: Lippincott Williams and Wilkins.

Arthur, W. H. (1974). Histology Text book. J.B. Lippincott Company Philadelphia and Toronto. 7th edition.

Bilkei-Gorzo, A. (1993). Neurotoxic effect of enteral Aluminium. Food Chem Toxicol.; 31:357–361.

Buraimoh, A.A. & Ojo, S.A. (2012). Effects of Aluminium Chloride Exposure on The Histology of Kidney of Wistar Rats. International Journal of Biology, Pharmacy and Allied Sciences; 1(11):1556-1568.ISSN: 2277-4998.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2011a). Effects of oral administration of aluminium chloride on the histology of the hippocampus of wistar rats. Curr. Res. J. Biol. Sci., 3: 509-515.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2011b). Behavioural endpoints of adult wistar rats, following aluminium chloride exposure. Br. J. Pharmacol. Toxicol ; 2: 273-276. ISSN: 2044-2467.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2011c). Effects of Aluminium Chloride on Anxiety-Related Behaviour. American Journal of Neuroscience, Science Publication; 2(2): 65-69, ISSN 1948-9900.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2012a). Effects of Aluminium Chloride Exposure on the Histology of the Cerebral Cortex of Adult Wistar Rats. Journal of Biology and Life Science@Macrothink Institute; Vol.3, No.1. ISSN 2157-6076. DOI: 10.5296/jbls.v3i1.1421.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2012b). Histological Study of the Effects of Aluminium Chloride Exposure on the Testis of Wistar Rats. American International Journal of Contemporary Research; Vol. 2, No. 5.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2012c). Effects of Aluminium Chloride Exposure on the Sperm Count of Adult Male Wistar Rats. Asian J. Biol. Sci; Vol.3 (2): 435-438.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. & Adebisi, S.S. (2012d). Aluminium Chloride Exposure Had No Effects on the Epididymis of Wistar Rats. American Medical Journal; Vol.3 (2):210-219. Science Publication.

Clayton, D.B. (1989). Water pollution at Lowermoore North Cornwall: Report of the Lowermoore incident health advisory committee. Truro, Cornwall District Health Authority, pp.22.

"Duodenal Anatomy" http:// emedicine.medscape.com/article/1898874-overview.

Exley, C.A. (1999). Molecular mechanism of Aluminium induced Alzheimer's disease. J. Inorg. Biochem., 76: 133-140.

Exley, C. (2005). The aluminium-amyloid cascade hypothesis and Alzheimer's disease aluminium and β-amyloid. Alzheimer's Disease, 38: 225-234. DOI: 10.1007/0-387-23226-5_11.

Garruto, R.M. & Brown, P. (1994).Tau protein, Aluminium, and Alzheimer's disease. Lancet.; 8904:989–993.

Grant, J.C.B., Basmajian, J.V. & Slonecker, C.E. (1989).*Grant's Method of Anatomy: A Clinical Problem-Solving Approach*. 11th Ed. London, UK: Williams and Wilkins.

Gray, H. & Lewis, W.H. (2000). *Gray's Anatomy of the Human Body*. 20th Edition. New York, NY: Bartleby.

Greger, J. L., Goetz, W. & Sullivan, D. (1985). Aluminium levels in foods cooked and stored   in aluminium pan, trays and foil. J. food prot 48: 772-777.

Guillaume, J., Praxis P., Sadasivam K., Pierre, B. & Robert, M. (2001). *Nutrition and Feeding of Fish and Crustaceans*. Springer. p. 31. ISBN 1-85233-241-7, 9781852332419.

Kloppel, H., Fliedner, A. & Kordel, W. (1997). Behaviour and endotoxicology of aluminium in soil and water. Review of the scientific literature. Chemosphere., 35: 353-363.

Latunde-Dada, G.O., Van der Westhuizen, J. & Vulpe, C.D. (2002). "Molecular and functional roles of duodenal cytochrome B (Dcytb) in iron metabolism". *Blood Cells Mol. Dis.* 29 (3): 356–60. doi:10.1006/bcmd.2002.0574. PMID 12547225.

Mari, S., Golub, & Stacey, L. (2001). Long-term consequences of developmental exposure to Aluminium in a suboptimal diet for growth and behavior of Swiss Webster mice. Neurotoxicology and Teratology.; 23:365–372.

Muller, G., Bernuzzi, V., Desor, D., Hutin, M.F., Burnel, D. & Lher, P.R. (1990). Developmental alteration in offspring of female rats orally intoxicated by Aluminium lactate at different gestation periods. Teratology.; 42:253–261.

Newairy, A.S., Salama, A.F., Hussien, H.M. & Yousef, M.I. (2009). Propolis alleviates aluminium-induced lipid peroxidaion and biochemical parameters in male rats. Food Chem Toxicol. 47(6):1093-8.

Roberts, E. (1986). Alzhermer's disease may begin in the nose and may be caused by aluminosilieates. Neurobiol Aging.; 7:561–567.

Schmidt, M.L., Zhukavera,V., Perl, D.P., Sheridan, S.K. & Schuck, T. (2001). Spinal cord neurofibrillary pathology in alzheimer disease and guam parkinsonism-dementia complex. J. Neuropathol. Exp. Neurol., 60: 1075-1086.

Sherwood, L. (2006). *Fundamentals of physiology: a human perspective* (Third edition). Florence, KY: Cengage Learning. pp. 768. ISBN 0-534-46697-4.

Solomon, B., Koppel, R. & Jossiphov, J. (2001). Immunostaining of calmodulin and Aluminium in Alzheimer's disease-affected brains. Brain Res Bull.;55:253–256.

Solomon, A., Gupta, P.K., LiVolsi, V.A. & Baloch, Z.W. (2002). Biology Sixth Edition, Brooks-Cole/ Thomson Learning ISBN 0-03-033503-5.

Somova, L.I., Missankov, A. & Khan, M.S. (1997). Chronic Aluminium intoxication in rats: dose dependent morphological changes. Methods Find Exp. Clin. Pharmacol., 19: 599-604.

Tahara, H. (2004). Osteomalacia and vitamin D deficiency in hemodialyzed patients. Clinical Calcium. 14:42–45.

van Gijn, J. & Gijselhart, J.P. (2011). "Treitz and his ligament." *Ned Tijdschr Geneeskd.* 155 (8). PMID 21557825.

WHO, (1997). Aluminium. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 194.

Yokel, R.A. (1985). Toxicity of gestational Aluminium exposure to the maternal rabbit and offspring. Toxicol Appl Pharmacol.;79:121–133.

Yokel, R.A. (2000).The toxicology of Aluminium in brain:review, Neurotoxicology .21 (5): 813-828.