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Thermocol plastination of ulcers in tongue and abomasum

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ABSTRACT

The present study was an attempt to plastinate animal tissues for demonstration of pathological lesions in bovine tissues. In the present study, bovine samples of abomasum (n=6) and tongues (n=6), exhibiting ulcers, were plastinated using economical thermocol-chloroform impregnation, instead of costly resins. Initially, tissues were fixed in 5% formol saline; dehydrated in pure acetone; impregnated in 15% thermocol in organic solvent and finally, cured by touchwood. Colour of pathological lesions became prominent in plastinated tissues. There was no change in texture of tissues, whereas the consistency of tissues became harder. At the end of plastination, reduction in mean mass and mean volume in tongue was 40.97% and 40.47% respectively. Whereas in abomasum, mean mass was reduced by 50.80% and mean volume by 46.61%. Despite reduction in mean mass and volume, plastinated tissues exhibited the gross alterations satisfactorily and tissues remained well preserved in dry state which was easy to handle, non-toxic and odourless**.** It was concluded that seven days fixation was sufficient for fixation of ulcers and thermocol solution can be used for impregnation process which was also suitable for histopathological analysis. The present approach of economical plastination is effective approach for dry preservation of ulcers in tongue and abomasum.

Keywords: Abomasum, Pathology, Plastination, Preservation, Teaching aids, Tongue, Thermocol

Plastination of gross pathological lesions is a vital tool for preservation of lesions for demonstration in teaching as well as for long term archiving of dry tissues with pathological lesions. Traditionally, the animal tissues are preserved in formaldehyde-based solutions (Dawson *et al*. 1990). However, it has serious disadvantages due to emission of harmful vapours of formaldehyde that has offensive odour and lead to irritation of eyes and skin. Furthermore, specimen jars are essential for the preservation of specimens that require maintenance in order to avoid fluid cloudiness that prevents the detail. Further, health hazards like carcinogenicity and contact dermatitis (Jadhav *et al*. 2016) and hypersensitivity (Dimenstein 2009) have also been reported with traditional tissue preservation methods. It has been claimed that formaldehyde's high solubility in water induces absorption in the respiratory and gastrointestinal pathways, resulting in nasal tumors in rodents (Merk and Speit 1998).

In the present study, new economical thermocol plastination technique has been demonstrated in order to overcome the drawbacks of traditional approach of organ preservation, which is a process of tissue preservation by embedding tissues in synthetic material wherein water and lipid in tissues are replaced by thermocol solution. Traditionally, in plastination, costly resins are used viz. silicon, epoxy and polyester where they replaced water and lipid from specimen by forced vaccum impregnation (Mahajan *et al*. 2016). Though, an economical method of plastination, incorporating recycled environment pollutants such as tea cups and thermocol for plastination of tissues at normal atmospheric pressure and at room temperature, has been reported to preserve organs (Mutturaj *et al*. 2014).

However, to preserve gross pathological lesions on tongue and abomasum, economical thermocol plastination approach has, so far, not been implemented by any Veterinary Pathology Department. Therefore, this pioneering attempt, to demonstrate ulcers in bovine tongue and abomasum, by plastination, using economical thermocol, in place of costly resins was undertaken with the objective of initial standardization of the process and to study the impact of plastination on bovine tissue, as well as lesions therein and also whether histopathological study is feasible on plastinated tissue.

MATERIALS AND METHODS

Collection of specimens: During post mortem examinations conducted at Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, tongue (n=6) and abomasum (n=6) of bovines were collected. Six samples of tongue and abomasum were abbreviated as TO1, TO2, TO3, TO4, TO5, TO6 and AB1, AB2, AB3, AB4, AB5, AB6 respectively. All samples of tongue and abomasum showed ulcers on it.

Preparation of tissue samples: Unwanted tissue and fat from the specimens was removed and all tissues were washed in running tap water for proper cleaning and to

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remove blood clots.

Fixation: All tissue samples were fixed in 5% formol saline. One litre of 5% formol saline was prepared by dissolving 9 g of sodium chloride and 50 ml pure formalin (40% formaldehyde) in 950 ml of tap water. Quantity of fixative used was 10 times more than volume of specimens. TO1, TO2, AB1 and AB2 were fixed for 7 days; TO3, TO4, AB3 and AB4 were fixed for 10 days and TO5, TO6, AB5 and AB6 were fixed for 15 days.

Dehydration: After washing in running tap water for 5 h, dehydration of the tissue samples was carried out by using 100% acetone (with volume of tissue and acetone used in the ratio of 1:5). Three changes of pure acetone were given to all tissue samples at room temperature. First treatment of acetone solution was given for three days; second treatment of acetone was given for seven days and finally, third treatment of acetone was given for another one week. Concentration of acetone was recorded by Alcoholometer and if concentration of acetone was reduced to less than 98% during third change, it was shifted to fourth change and extended till concentration of acetone remained more than or equal to 98% for two consecutive days.

Impregnation: Dehydrated tissue samples of tongue and abomasum were air dried for 15 min. Thereafter, thermocol solution was prepared by dissolving 15 g of thermocol along with 5 g of petroleum jelly per 100 ml of chloroform and kept for half an hour so that petroleum jelly and thermocol dissolves well in chloroform. No forced impregnation was carried out rather, it was done at room atmospheric pressure and at room temperature. Complete immersion of tongue and abomasum in impregnation solution indicates completion of impregnation (Ramakrishna and Leelavathy 2019).

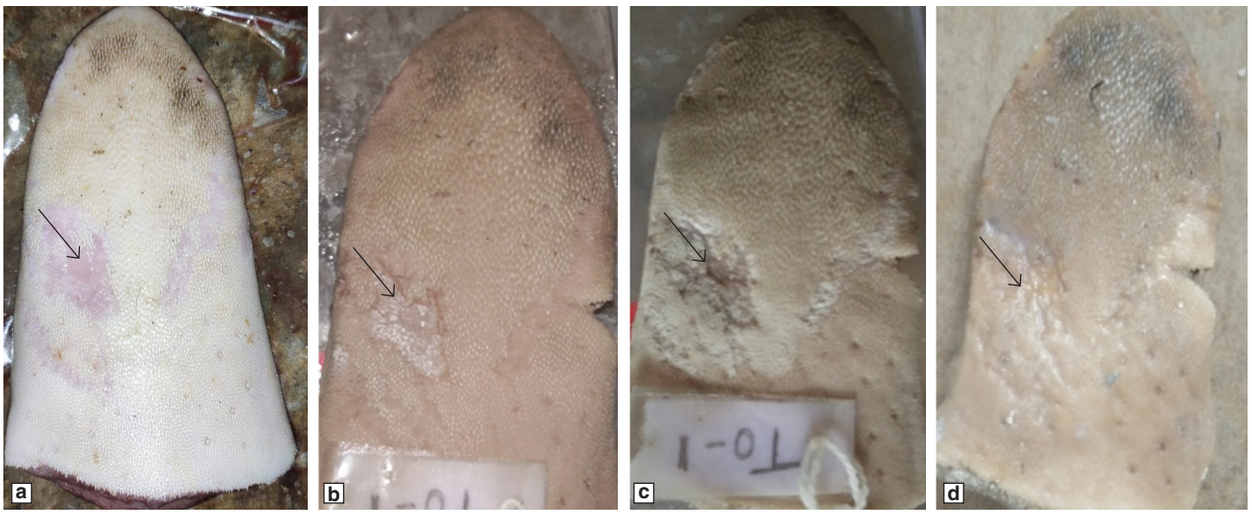
Curing: Excess impregnation solution was mopped up and all tissue samples were air dried at room temperature. For curing of tongue and abomasum tissue samples, touchwood was used, twice, at an interval of 24 h. Thereafter, tissue samples were air dried at room temperature till its complete drying.

Study of alterations in tongue and abomasum due to plastination: Colour, consistency, texture, mass and volume of fresh tissue samples were recorded at the time of collection. Thereafter, these parameters were recorded at every stage of plastination. Colour was recorded by taking digital pictures, consistency and texture were felt by tactile sensation. Mass was measured on a digital weighing machine whereas volume was measured by fluid displacement method.

Histopathological studies: Histopathological alterations were studied in impregnated tissues wherein impregnated tissue was directly embedded in paraffin wax avoiding dehydration and clearing step. Then blocks were made. Sections of tissues with thickness of 5 µm were cut and stained by routine Hematoxylin and Eosin-Phloxine (H&E-Phloxine) method (Culling *et al*. 1974) and observed under light microscope. The histopathological alterations in

Table 1. Mass, volume, colour, consistency and texture of fresh tongue and abomasum and their lesions

Case No.	Gross lesions	Mass (g)	Volume (ml)	Colour of tissue	Colour of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
TO1	Ulcers	56.00	58	Pinkish white	Pink	Soft	Slightly hard	Rough	Rough
TO2	Ulcers	102.00	96	Pinkish white	White	Soft	Soft	Rough	Irregularly depressed surface
TO3	Ulcers	150.00	138	Pinkish white	Pink	Soft	Slightly hard	Rough	Rough
TO4	Ulcers	105.00	101	Pinkish white	Pink	Soft	Soft	Rough	Irregular raised surface
TO5	Ulcers	90.00	105	Pinkish white	Pink	Soft	Slightly hard	Rough	Rough
TO6	Ulcers	115.00	107	Pinkish white	Pink	Soft	Soft	Rough	Rough
AB1	Ulcers	217.15	200	Creamish	Blackish brown	Soft	Slightly hard	Smooth elongate raised mucosal folds	Granular, slightly rough
AB2	Ulcers	176.25	132	Creamish	Blackish brown	Soft	Slightly hard	Smooth elongate raised mucosal folds	Slightly rough
AB3	Ulcers	146.11	117	Creamish	Blackish brown	Soft	Slightly hard	Slightly rough	Slightly rough
AB4	Ulcers	164.83	105	Creamish	Blackish brown	Soft	Slightly hard	Smooth elongate raised mucosal folds	Granular, slightly rough
AB5	Ulcers	99.10	115	Creamish	Blackish brown	Soft	Slightly hard	Slightly rough	Granular, slightly rough
AB6	Ulcers	93.95	104	Creamish	Blackish brown	Soft	Slightly hard	Smooth elongate raised mucosal folds	Slightly rough



Ulcers in fresh tongue **Ulcers in fixed tongue** **Ulcers in dehydrated tongue** **Ulcers in cured tongue**

Fig. 1. Tongue ulcers at fresh **(a)**, fixation **(b)**, dehydration **(c)** and curing **(d)** stage.

Table 2. Mass, volume, colour, consistency and texture of fixed tongue and abomasum and their lesions

Case No.	Gross lesions	Mass (g)	Volume (ml)	Colour of tissue	Colour of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
TO1	Ulcers	54.04	58	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
TO2	Ulcers	98.53	95	Light brown	White	Slightly hard	Slightly harder	Rough	Irregularly depressed surface
TO3	Ulcers	144.00	136	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
TO4	Ulcers	100.90	99	Light brown	Brown	Slightly hard	Slightly harder	Rough	Irregular raised surface
TO5	Ulcers	86.67	103	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
TO6	Ulcers	110.86	105	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
AB1	Ulcers	215.00	198	Creamish	Blackish brown	Slightly hard	Slightly harder	Smooth elongate raised mucosal folds	Slightly rough
AB2	Ulcers	175.00	131	Creamish	Blackish brown	Slightly hard	Slightly Harder	Smooth elongate raised mucosal folds	Slightly rough
AB3	Ulcers	147.00	115	Creamish	Blackish brown	Slightly hard	Slightly Harder	Slightly rough	Slightly rough
AB4	Ulcers	166.00	103	Creamish	Blackish brown	Slightly hard	Slightly Harder	Smooth elongate raised mucosal folds	Slightly rough
AB5	Ulcers	100.00	112	Creamish	Blackish brown	Slightly hard	Slightly harder	Slightly rough	Slightly rough
AB6	Ulcers	95.00	102	Creamish	Blackish brown	Slightly hard	Slightly harder	Smooth elongate raised mucosal folds	Slightly rough

impregnated tissues were compared with histopathological alterations in tissue samples collected from the same tongue (TO1, TO2, TO3, TO4, TO5 and TO6) and abomasum (AB1, AB2, AB3, AB4, AB5 and AB6) tissue samples in 10% formalin at time of collection that were processed by traditional method of tissue processing.

Statistical analysis: The observations were recorded on the same tissue samples in fresh condition as well as after treatment in plastinated condition, therefore paired t-test was used to test any significant difference ($p<0.05$) in mean mass and mean volume between fresh and plastinated samples. Statistical Package for the Social Sciences 17.0 software was used for statistical analysis.

RESULTS AND DISCUSSION

Unplastinated tissue: The mass, volume, colour (Figs 1a, 2a), consistency and texture of fresh tongue and abomasum tissue samples exhibiting gross lesions are shown in Table 1.

Fixed tissue: After fixation of tongue (Fig. 1b) and abomasum (Fig. 2b) tissue samples, it was seen that all tissues exhibited loss of pink colour. This was due to loss of blood from specimens through diffusion into surrounding medium (Ameko *et al*. 2012). All tissue samples of tongue and abomasum were found to be fixed properly in 7 days. After fixation, mass and volume was reduced in all tongue and abomasum tissues (Table 2). The consistency of tissues and lesions therein became harder and no alteration was observed in texture of tissues and their lesions.

Dehydrated tissues: Bovine tongue samples got dehydrated within 12 days and abomasum tissue samples took 13 days for dehydration (Supplementary Table 1). However, during dehydration, mass and volume of fixed tissues was further reduced (Table 3). In all tongue (Fig 1c) and abomasum (Fig 2c) tissue samples, colour of fixed tissues and their lesions was maintained after dehydration. This indicated that duration of fixation was sufficient to fix gross lesions on tongue and abomasum.

Guhr *et al.* (1987) used ascending graded of ethanol followed by three changes of concentrated acetone and finally 100% methylene chloride for effective dehydration. However, in present study, proper dehydration was achieved by using only acetone.

Thermocol plastinated tongue and abomasum tissue samples: The colour of all tongue and abomasum (Fig. 2d) tissue samples became darker during impregnation that reverted back to light colour during curing, thereby revealing the lesions therein, prominently (Fig. 1d, 2e). In present study, it was reported that, during impregnation, tongue and abomasum samples floated initially and later got immersed in the impregnation solution. Finally, tongue samples sank gradually to the bottom of impregnation solution in 43 to 46 days and abomasum tissue samples in 48 to 52 days indicating completion of impregnation. It was found that impregnation was complete only when they were properly dipped in impregnation solution. Ramkrishna and Leelavathy (2019) reported that 2-4 weeks duration was

Table 3. Mass, volume, colour, consistency and texture of dehydrated tongues and abomasum and their lesions

Case No.	Gross Lesions	Mass (g)	Volume (ml)	Color of tissue	Colour of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
TO1	Ulcers	42.48	46	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
TO2	Ulcers	74.81	73	Light brown	White	Slightly hard	Slightly harder	Rough	Irregularly depressed surface
TO3	Ulcers	113.52	107	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
TO4	Ulcers	78.90	75	Light brown	Brown	Slightly hard	Slightly harder	Rough	Irregular raised surface
TO5	Ulcers	68.49	82	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
TO6	Ulcers	86.69	80	Light brown	Brown	Slightly hard	Slightly harder	Rough	Rough
AB1	Ulcers	165.46	153	Creamish	Blackish brown	Slightly hard	Slightly harder	Smooth elongate raised mucosal folds	Slightly rough
AB2	Ulcers	138.25	101	Creamish	Blackish brown	Slightly hard	Slightly harder	Smooth elongate raised mucosal folds	Slightly rough
AB3	Ulcers	113.13	86	Creamish	Blackish brown	Slightly hard	Slightly harder	Slightly rough	Slightly rough
AB4	Ulcers	131.21	78	Creamish	Blackish brown	Slightly hard	Slightly harder	Smooth elongate raised mucosal folds	Slightly rough
AB5	Ulcers	75.92	82	Creamish	Blackish brown	Slightly hard	Slightly harder	Slightly rough	Slightly rough
AB6	Ulcers	74.10	80	Creamish	Blackish brown	Slightly hard	Slightly harder	Smooth elongate raised mucosal folds	Slightly rough

sufficient for impregnation which was found to be merely indicative of initial immersion in the present study. Though consistency of plastinated tissues became hard after curing, there was no alteration in texture of plastinated tissues (Table 4). The mass and volume of dehydrated tongue and abomasum tissue samples were further reduced.

Quantitative analysis of alterations in weight and volume due to plastination of tongue and abomasum: It was found that mean mass and mean volume of fresh tongue and abomasum samples, at the stage of collection, reduced significantly by end stage of plastination. Reduction in mean mass and mean volume in tongue was 40.97% and 40.47%, respectively, whereas in abomasum, mean mass was reduced by 50.80% and mean volume by 46.61%.

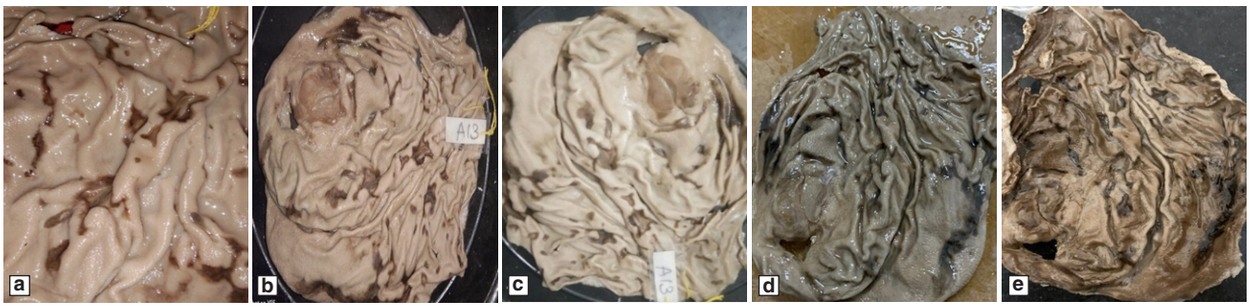
The decrease in weight and volume of plastinated tissue samples in our study did not significantly impact the study of gross lesions on tongue and abomasum tissue samples. Thus, plastination by this novel economical approach proved suitable for studying gross pathological lesions in animal tissues.

Histopathological studies: The processing of impregnated tissues revealed preservation of morphological architecture of tissue sections. Comparison of histopathological analysis of ulceration in tongue tissue impregnated in thermocol solution with corresponding tongue tissues processed as per routine approach revealed, superficial necrosis along with infiltration of mononuclear cells (Fig. 3a), and impregnated abomasum tissue showing sloughing of superficial layer with mild mononuclear cell infiltration (Fig. 4a) and congestion along with focal to diffuse mononuclear cell infiltration in submucosal layer. Comparison of histopathological observations in slides of impregnated tissues with slides of routine histopathological preparations of the same tissue collected before processing of the tissues for plastination, revealed that slides of impregnated tissues were more intensely stained and were prominent than the slides of fresh tissue samples (Figs 3b, 4b) despite the fact that both types of slides were stained with the same Hematoxylin and Eosin stain.

No study has been reported in the published literature for preservation of pathological lesions in tongue and abomasum by the economical method used in the present study. Most studies on plastination of normal tissues have largely used costly resins, viz. silicon, epoxy, polyester (Hagens *et al.* 1987, Stoyanav *et al*. 2015, Hayat *et al*. 2018, Tote and Tote 2020). Epoxy resins commonly used by the plastinators are well recognized as skin, eye and mucous membrane irritants (Holladay *et al.* 2001). Further, such approaches have not conducted detailed studies of alterations in mass, volume, colour, consistency etc. as undertaken in the present study. Therefore, the economical protocol of tissue plastination by using thermocol solution at room atmospheric pressure and room temperature that renders satisfactory preservation of colour and texture of pathological lesions can be considered as superior to the costly and hazardous resins used in a procedure involving vaccum conditions and cold temperature rendering the

Table 4. Mass, volume, colour, consistency and texture of plastinated tongues and abomasum and lesions therein

Case No.	Gross lesions	Mass (g)	Volume (ml)	Colour of tissue	Colour of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
TO1	Ulcers	36.11	39	Light Brown	Brown	Hard	Hard	Rough	Rough
TO2	Ulcers	62.09	60	Light Brown	Creamish	Hard	Hard	Rough	Irregularly depressed surface
TO3	Ulcers	93.08	90	Light Brown	Brown	Hard	Hard	Rough	Rough
TO4	Ulcers	67.86	61	Light Brown	Brown	Hard	Hard	Rough	Irregular raised surface
TO5	Ulcers	59.59	69	Light Brown	Brown	Hard	Hard	Rough	Rough
TO6	Ulcers	73.26	65	Light Brown	Brown	Hard	Hard	Rough	Rough
AB1	Ulcers	113.34	119	Creamish	Blackish brown	Hard	Hard	Smooth elongate raised mucosal folds	Slightly rough
AB2	Ulcers	98.79	81	Creamish	Blackish brown	Hard	Hard	Smooth elongate raised mucosal folds	Slightly rough
AB3	Ulcers	77.72	66	Creamish	Blackish brown	Hard	Hard	Slightly rough	Slightly rough
AB4	Ulcers	90.54	63	Creamish	Blackish brown	Hard	Hard	Smooth elongate raised mucosal folds	Slightly rough
AB5	Ulcers	56.94	65	Creamish	Blackish brown	Hard	Hard	Slightly rough	Slightly rough
AB6	Ulcers	55.58	63	Creamish	Blackish brown	Hard	Hard	Smooth elongate raised mucosal folds	Slightly rough



Ulcers in fresh abomasum | Ulcers in fixed abomasum | Ulcers in dehydrated abomasum | Ulcers in impregnated abomasum | Ulcers in cured abomasum

Fig. 2. Abomasal ulcers at fresh **(a)**, fixation **(b)**, dehydration **(c)**, impregnation **(d)** and curing **(e)** stage.

plastination of tissue economically unviable especially for all the developing countries. Therefore, we suggest that, this economical technique can be usefully applied in preservation of pathological tissues. This novel economical thermocol-chloroform plastination approach of tissues preserves colour, consistency and texture of gross pathological lesions wherein the reduction in weight and volume of the tissue does not interfere in observation of gross pathological lesions therein. Further, with feasibility of histopathological analysis of plastinated tissues, this approach is highly useful for teaching pathological alterations. The plastinated tissues being dry, hazard-free preparations that are highly convenient to transport.

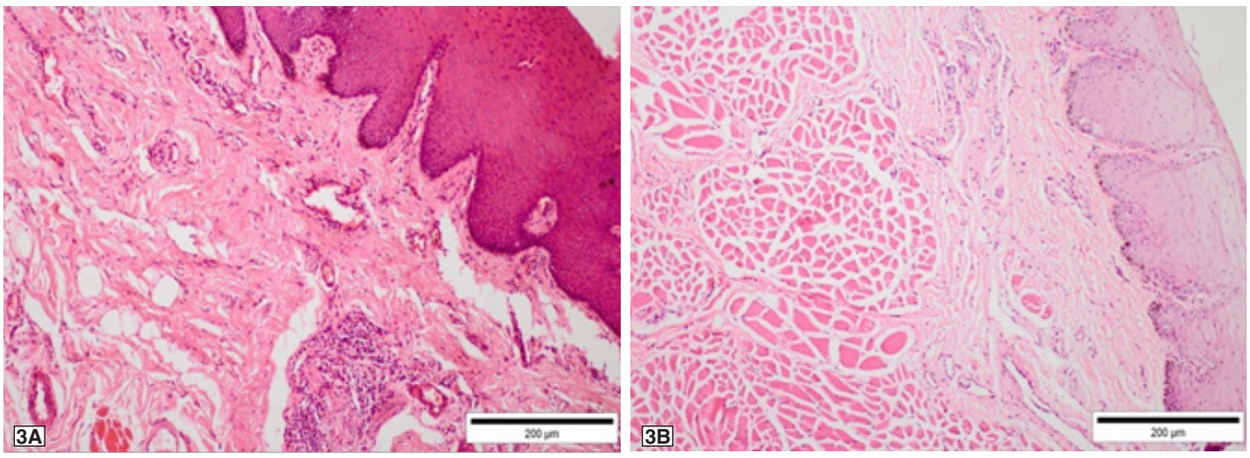


Fig. 3. Comparison of mild subepithelial necrosis along with infiltration of mononuclear cells in impregnated tissue of tongue after embedding in wax **(A)**, with fresh tissue after routine processing **(B)**. (H&E, Bar=200µm)

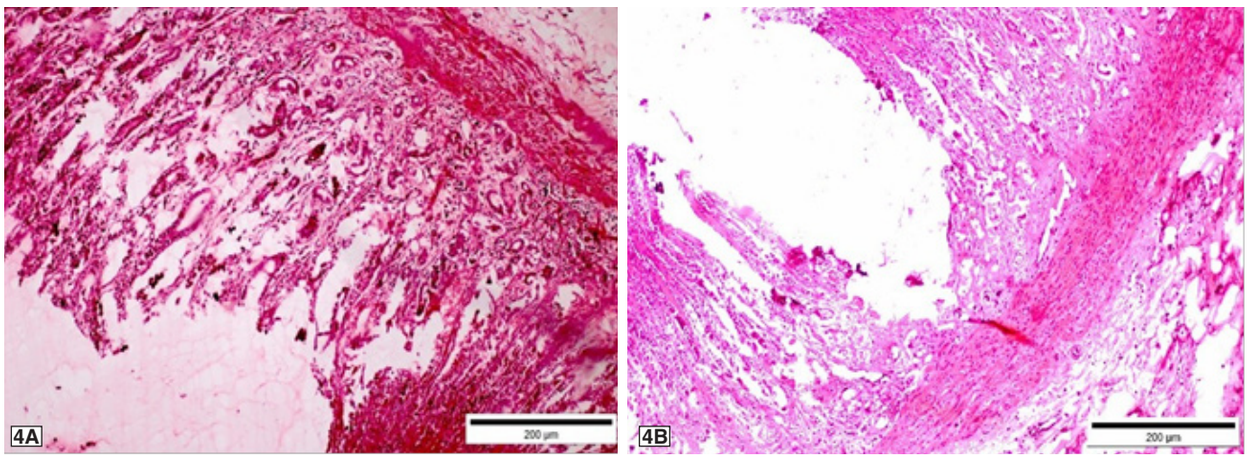


Fig. 4. Comparison of sloughing of superficial layer with mild mononuclear cell infiltration cells in impregnated tissue of abomasum after embedding in wax **(A)**, with fresh tissue after routine processing **(B)**. (H&E, Bar=200µm)

REFERENCES

Ameko E, Achio S, Alhassan S, Adasu C, Dzagbletey E T and Abbey P R. 2012. Plastination of some cow and ram organs in Ghana for use as teaching aids. *International Journal of Pure and Applied Sciences and Technology* **8**(1): 57–68.

Culling C F A. 1974. Basic staining and mounting procedures. *Handbook of Histopathological and Histochemical Techniques*. 3rd edn. Butterworths and Co, London. pp. 192-200.

Dawson T P, James R S and Williams G T. 1990. Silicone plastinated pathology specimens and their teaching potential. *Journal of Pathology* **162**(3): 26572.

Dimenstein I B. 2009. A pragmatic approach to formalin safety in anatomical pathology. *Laboratory Medicine* **40**(12): 740–46.

Guhr A, Mueller A, Anton H, Hagens G V and Bickley H C. 1987. Complete examination of mastectomy specimens using sheet plastination with epoxy resin. *Journal of the International Society for Plastination* **1**(1): 23–27.

Hayat K, Qureshi A S, Rehan S and Rehman T. 2018. Plastination-an innovative preservative technique in anatomy. *Trends in Anatomy and Physiology* **1**(1): 003.

Holladay S D, Blaylock B L and Smith B J. 2001. Risk factors associated with plastination: I. Chemical toxicity considerations. *Journal of the International Society for Plastination* **16**: 9–13.

Jadhav A, Kulkarni P R and Chakre G. 2016. Plastination: A novel way of preserving tissues. *Al Ameen Journal of Medical Science* **9***(*4**):** 212–14.

Mahajan A, Agarwal S, Tiwari S and Vasudeva N. 2016. Plastination: An innovative method of preservation of dead body for teaching and learning anatomy. *MAMC Journal of Medical Sciences* **2**(1): 38–42.

Merk O and Speit G. 1998. Significance of formaldehyde-induced DNA–protein crosslinks for mutagenesis. *Environmental and Molecular Mutagenesis* **32**(3): 260–68.

Mutturaj R P, Prasad R V, Jamuna K V, Murty C V and Ramkrishna V. 2014. Plastination of specimens by recycling environmental pollutants. *Indian Journal of Veterinary Pathology* **26**(2): 130–31.

Ramkrishna V and Leelavathy N. 2019. The use of waste plastics for plastination of organic materials and in civil construction materials. *Waste Management and the Environment IX*. 231, pp. 193.

Stoyanav J, Georgieva A and Sivrev D. 2015. Use of physical and chemical factors in the development of plastination anatomical preparations. *Trakia Journal of Sciences* **13**(2): 21–22.

Tote D and Tote S. 2020. Comparative study between various reagents of plastination in making museum specimen. *International Journal of Current Research and Review* **12**(22): 126.

Von Hagens G, Tiedemann K and Kriz W. 1987. The current potential of plastination. *Anatomy and Embryology* **175**(4): 411–21.