RESEARCH ARTICLE

Evaluation of Bubaline Derived Composite Collagen Glue for Enhanced Healing and Cosmetic Efficacy of Aural Haematoma in Dogs

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Abstract

The objective of the present study was to evaluate collagen glue for the treatment of aural haematoma in dogs. Eighteen clinical cases of dogs suffering from aural haematoma were divided into three groups. In group I (n=6), horizontal mattress sutures were applied, in group II (n=6) horizontal mattress sutures along with collagen glue was applied and in group III (n=6) sole application of collagen glue was done for the obliteration of dead space. The cosmetic appearance of ear pinna was compared in all the groups after complete healing. Haemato-biochemical parameters recorded revealed that the most values were within normal physiological limits without group or period effect, except for total leukocyte count and neutrophils (%) which significantly significant in all groups from day 0 to day 7 post-treatment and the values were also lower in group III than group I. Histological observations concluded that collagen glue treated group showed reduced inflammatory cells, fast proliferation of fibroblasts, early epithelialization and improved neovascularization along with significant collagen deposition and arrangement.

Key words: Aural haematoma, Collagen glue, Dog, Histopathology, Horizontal mattress suture.

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Introduction

An aural haematoma is a blood-filled subcutaneous fluctuant swelling on the pinna resulting from the rupture of capillaries and subsequent separation of the auricular cartilage and underlying skin (Lahiani and Niebauer, 2019). It occurs as a result of constant ear shaking and rubbing, often secondary to underlying conditions such as otitis, ectoparasitism, ottorrhoea, foreign bodies, hypersensitivity and allergic dermatitis (Safwan *et al*., 2018). Aural haematoma is very common ear affection in dogs. The incidence of aural haematoma among other diseases is 0.25% (Neill *et al.,* 2021). The haematoma may be warm to touch with underlying erythematous skin containing a sero-haemorrhagic fibrotic rich fluid. During normal healing, the fluid resorbs and fibrosis occurs, the contraction of fibrotic tissue results in malformation of ear pinna (Hewitt and Bajwa, 2020).

The principle of surgical treatment is drainage and compression of cavity. Conservative treatment by fine-needle aspiration, instillation of corticosteroids and suture application has shown complications like incomplete healing, pinna thickening, infections, necrosis of the pinna, long surgical duration and recurrence (Lahiani and Niebauer, 2019). The latest modalities for aural haematoma are tissue adhesives such as collagen glue; cyanoacrylates (Dermabond, Omnex), albumin glutaraldehyde adhesives (BioGlue) and fibrin glues (Tisseel) are the most widely used commercial adhesives. Unfortunately, the high cytotoxic effect of cyanoacrylate or aldehyde containing products especially as tissue adhesives cannot be ignored (Yang *et al*., 2021). The characteristics of collagen include good compatibility with living tissue, promotion of cell growth, absorption and assimilation of implants (Shimizu, 1977). It is the only matrix protein which supports both platelet adhesion and complete activation leading to haemostasis (Farndale *et al.,* 2004). Collagen glue could be thought to be an important tool in

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the treatment of aural haematoma in dogs for several reasons: minimally invasive, reduced recovery time, may cutdown the risk of anaesthesia, cost-eeffective, prevent air or body fluid leakage, localize delivery of biocidal agent and most importantly better cosmetic outcomes (Yang *et al.*, 2021). The current study was planned to evaluate the bubaline derived composite collagen glue for enhanced healing and cosmetic efficacy of aural haematoma in dogs.

Materials and Methods

The present study was conducted from 2022 to 2024 on 18 clinical cases of dogs suffering from aural haematoma presented in Department of Veterinary Surgery and Radiology, and Veterinary Clinical Complex of NDVSU, Mhow (Indore, India). These dogs were selected, irrespective of age, sex or breed and divided into three groups. Each dog underwent a clinical examination to assess the presence of ticks, lice, dermatitis, otitis and the extent of haematoma. The research proposal was approved by Institutional animal Ethics Committee (IAEC), according to the guidelines of CPCSEA.

Collagen Glue

Collagen-based medical adhesives and sealants have been derived from bubaline source, which is rich in Type I collagen. These soluble or partially fibrillar collagen monomers are chemically modified with an acylating agent, sulfonating agent or a combination prior to polymerization (Kelman and DeVore, 1993). Collagen is a major element of the extracellular matrix, and has many important roles in cellular attachment, cell division, cell movement and regeneration of organs. Implanted collagen is replaced by host tissue at the implantation site therefore it has attracted attention as an ideal biodegradable material (Sekine *et al.*, 2001). The composite collagen glue not only serves as a biomimetic and bioactive substance which provides structural support, facilitates faster cosmetic healing and regeneration, but it is also bio-compatible, bioactive, non-antigenic, non-toxic and acts as tissue filler in the tissue voids (Jain *et al.*, 2023) (Fig. 1).

Fig. 1: Composite acellular bubaline collagen glue

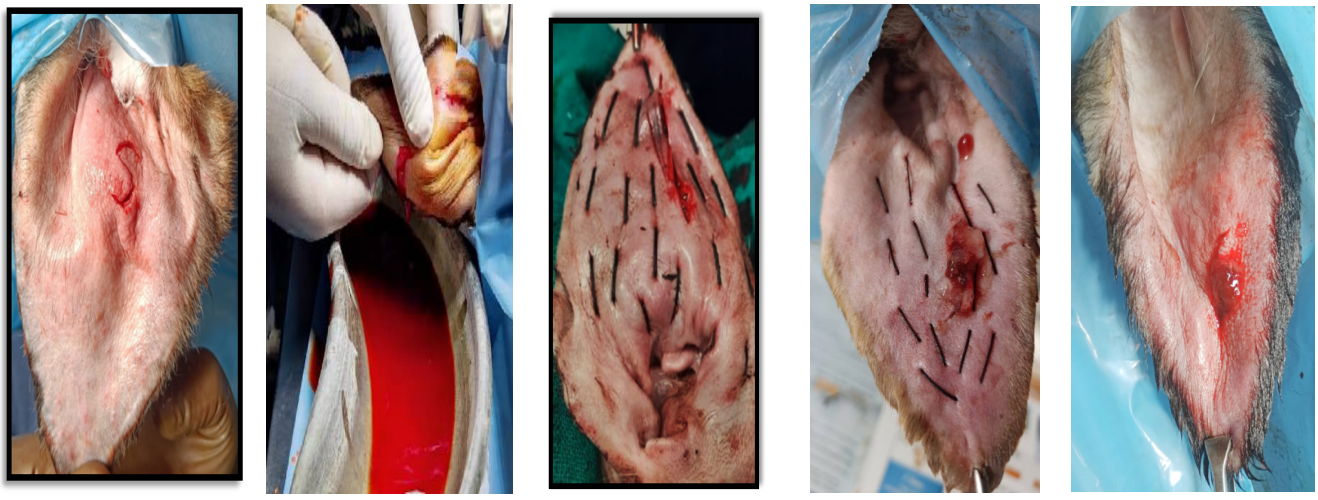
Anaesthesia

Prior to the surgery, dogs were fasted for 12 h and withheld water for 4 h. The surgical site was prepared aseptically. General anaesthesia was induced using a combination of Atropine sulphate @ 0.04 mg/kg b.wt., S/C, inj. Xylazine @ 1 mg/kg b.wt., I/M as preanesthetics and inj. Ketamine @ 10 mg/kg b.wt., I/M for induction of anaesthesia. Maintenance of anaesthesia was done using 1:1 ratio of Diazepam and Ketamine as required.

Surgical Technique

In all groups, S-shaped incision was made on the ventral surface of the pinna (Fig. 2a), followed by removal of blood clots and fibrous material (Fig. 2b). In group I, horizontal mattress sutures were applied (Fig. 2c). In group II, collagen glue along with horizontal mattress sutures were applied (Fig. 2d) and in group III, only collagen glue was applied (Fig. 2e).

Postoperative care included antiseptic dressing with povidone iodine and compression bandaging of the ear



(a) (b) (c) (d) (e)

Fig. 2: Steps of surgical technique: (a) S-shaped incision along the length of aural haematoma, (b) Draining of haematogenous fluid, (c) Application of horizontal mattress suture to in group I, (d) Application of horizontal mattress suture along with collagen glue in group II, (e) Application of collagen glue in group III

pinna, repeated every third day until suture removal in group I, II and complete healing in group III. Additionally, all groups receive antibiotic and anti-inflammatory medications. The efficacy of each treatment modality was assessed based on haematoma resolution and postoperative complications.

Parameters Studied

The cosmetic efficacy of ear pinna was evaluated after complete healing based on the parameters like ear pinna drooping, scar formation, suture embedding, fibrosis and distortion of ear pinna as per Safwan *et al*. (2018) with some modifications.

Various haematological parameters like, total erythrocyte count, haemoglobin concentration, packed cell volume, total leukocyte count, differential leukocyte count, and thrombocyte (Platelet) count were recorded using fully automatic blood cell counter (Diatron Model Abacus 380). Different biochemical parameters like total protein and albumin were recorded using commercially available kits (Erba Manheim), while globulin fraction was recorded by subtracting serum albumin from serum protein, albumin: globulin ratio (A/G ratio) was obtained by dividing serum albumin concentration with serum globulin concentration and fibrinogen was estimated as described by Millar *et al.* (1970).

The histopathological scoring for H & E stained sections was done based on parameters like presence of inflammatory cells, fibroblasts proliferation and neovascularization. The Van Gieson stained sections were scored based on parameters like collagen fibres density, arrangement and thickness. The data were analysed by using Completely Randomized Design (Snedecor and Cochran, 1994).

Results and Discussion

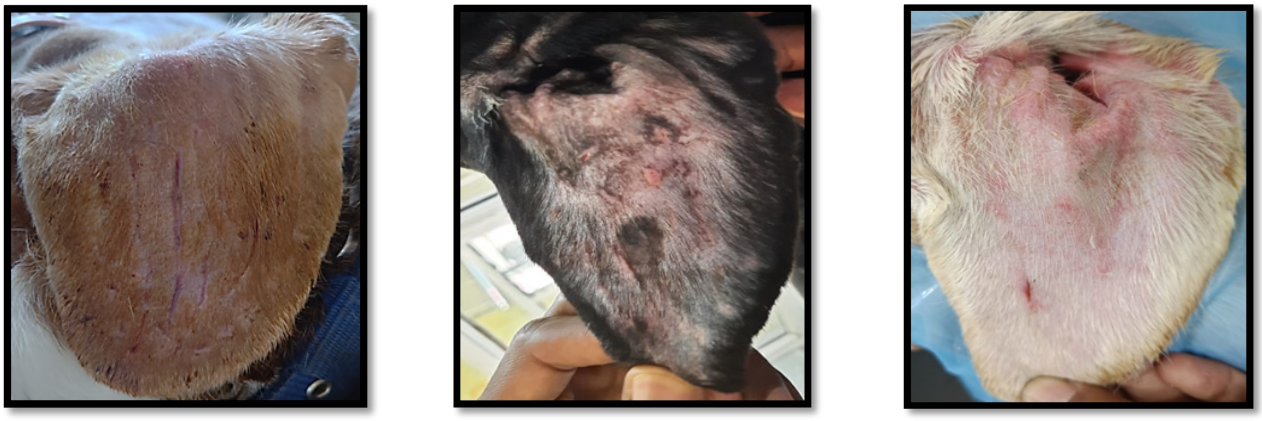
The best cosmetic appearance was shown by group III, with no complications (Fig. 3c). Group II showed moderate issues such as ear drooping and scarring (Fig. 3b), while group I had the poorest appearance with ear distortion and suture scarring (Fig. 3a). The order of best cosmetic appearance is group III > group II > group I (Table 1).

Table 1: Cosmetic appearance scores

	Groups		
Parameters	I	II	III
Ear pinna carriage	+	+	-
Embedding of suture	-	-	-
Scar formation	+	+	-
Fibrosis & Distortion of ear	+	-	-
Total score	+++	++	-

The erythrocytic count, haemoglobin concentration and packed cell volume were found to be unaffected at all-time intervals and fluctuated around the normal physiological values in all the groups of animals. On comparison among all the groups, the decreasing order of total leucocyte count ($x10^3/\mu L$) and neutrophils (%) was in group III <group II< group I (Table 2). The initial elevation by day 3rd may be attributed to infection and inflammatory response of the host to the biomaterial, anaesthesia or surgical stress, but it decreased by day 7th which indicates tolerance of host to the biomaterials. The percentage of neutrophils were high initially because of inflammatory response elicited by surgical trauma or implantation of foreign material whereas, in collagen glue treated group neutrophil percentage was lowest indicating anti-inflammatory nature of collagen (Shakya *et al.,* 2016). It indicates early wound re-epithelization and tissue repair which further leads to faster healing process. Neutrophils also produce a variety of growth such as interleukin-8 factors (Rennekampff *et al.,* 2000) and vascular endothelial growth factor (McCourt *et al.,* 1999) that could promote repair of injured tissue.

The lymphocytic, eosinophilic, monocytic and thrombocytic counts remained within normal physiological limits, showing non-significant fluctuations in all the



(a) (b) (c)

Fig. 3: Cosmetic appearance of ear pinna in different groups after complete healing. (a) Cosmetic appearance of ear flap in group I (Prominent scar formation at incision line and suture bites, (b) Cosmetic appearance of ear flap in group II (Scar formation at suture bites), (c) Cosmetic appearance of ear flap in group III (No Scar formation)

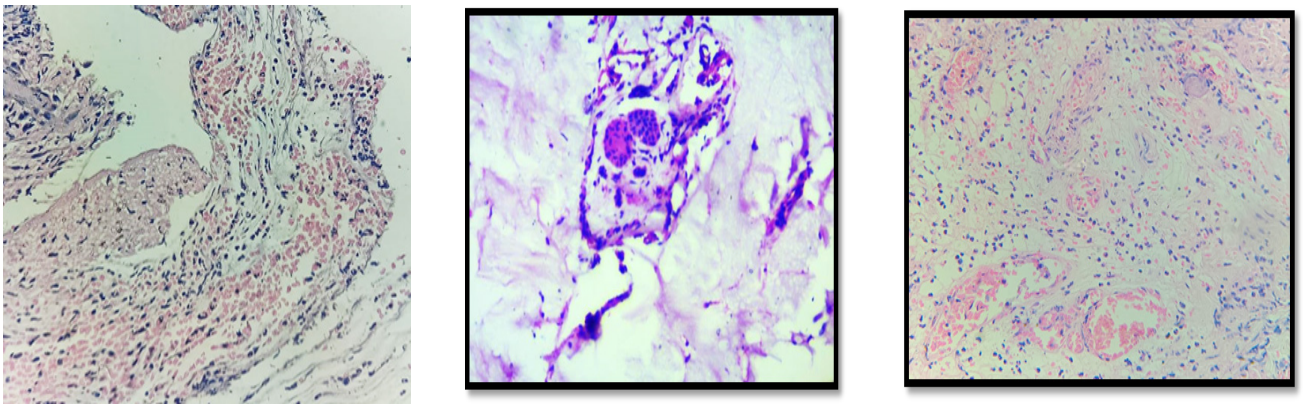
Table 2: Mean ± SE haemato-biochemical profile of dogs at different time intervals post-operatively in different groups

Blood Parameter	Groups	Post operative days		
		0	3rd	7th
Total erythrocyte count ($x10^6$/µL)	I	6.51±0.33	6.55±0.33	6.46±0.32
	II	6.55±0.28	6.43±0.31	6.56±0.29
	III	6.51±0.24	6.56±0.24	6.56±0.24
Haemoglobin (g/dL)	I	12.58 ±0.29	12.55±0.30	12.43 ± 0.31
	II	12.55 ± 0.23	12.48±0.23	12.48±0.23
	III	12.58 ± 0.21	12.55±0.16	12.58 ± 0.18
PCV (%)	I	39.86 ± 1.58	39.30 ± 1.25	39.75 ± 0.94
	II	38.86 ± 1.38	39.30 ± 1.25	39.91 ± 0.84
	III	39.65 ± 1.62	39.92 ± 1.69	39.93 ± 0.94
Total leucocyte count ($x10^3$/µL)	I	15.53^{b}±0.94	13.56^{ab}± 0.61	12.61^{a} ± 0.31
	II	15.45^{b} ±0.80	13.11^{ab} ± 0.73	11.38^{ab} ± 0.41
	III	14.45^{b}±0.89	13.38^{ab} ± 0.80	11.19^{a} ± 0.53
Neutrophils (%)	I	82.58^{a} ± 3.40	77.48^{b} ± 2.15	73.65^{b} ± 3.09
	II	81.57^{a}± 3.09	76.55^{b} ± 2.53	72.26^{b} ± 1.86
	III	81.23^{a}± 1.87	76.22^{b}± 3.13	71.91^{c}± 1.53
Lymphocytes (%)	I	15.49 ± 1.13	20.89 ± 0.65	24.99 ± 0.47
	II	16.43 ± 0.80	21.76 ± 0.80	25.77 ± 0.84
	III	17.25 ± 1.01	22.27 ± 1.18	26.18 ± 0.71
Monocytes	I	1.10 ± 0.49	0.97 ± 0.34	0.95 ± 0.17
	II	1.21 ± 0.33	1.12 ± 0.33	1.41 ± 0.33
	III	0.77 ± 0.31	1.00 ± 0.16	1.58 ± 0.00
Eosinophils	I	0.83 ± 0.21	0.66 ± 0.22	0.41 ± 0.23
	II	0.79 ± 0.22	0.57 ± 0.16	0.57 ± 0.16
	III	0. 75 ± 0.22	0.51 ± 0.21	0.33 ± 0.21
Platelets ($x10^5$/µL)	I	282.67 ± 16.10	296.33 ± 14.11	292.50 ± 14.11
	II	270.00 ± 21.11	300.33 ± 12.97	293.00 ± 12.18
	III	284.17 ± 11.85	287.50 ± 11.16	288.17 ± 9.87
Total protein (g/dL)	I	5.08 ± 0.17	5.07 ± 0.14	5.11 ± 0.19
	II	5.41± 0.24	5.59 ± 0.19	5.59 ± 0.19
	III	5.49 ± 0.16	5.61 ± 0.20	5.78 ± 0.15
Albumin (g/dL)	I	3.05 ± 0.13	3.11 ± 0.12	3.07 ± 0.14
	II	3.50 ± 0.20	3.67 ± 0.21	3.67 ± 0.21
	III	3.35 ± 0.17	3.45 ± 0.21	3.63 ± 0.27
Globulin (g/dL)	I	1.86 ± 0.07	1.96 ± 0.07	1.95 ± 0.12
	II	1.96 ± 0.11	1.92 ± 0.14	1.92 ± 0.14
	III	2.14 ± 0.04	2.16 ± 0.04	2.06 ± 0.09
A:G ratio	I	1.63 ± 0.03	1.58 ± 0.08	1.61 ± 0.13
	II	1.83 ± 0.20	1.98 ± 0.22	1.98 ± 0.22
	III	1.57 ± 0.10	1.60 ± 0.12	1.80 ± 0.23
Fibrinogen (mg/dL)	I	242.00 ± 10.29	244.83 ± 10.10	242.83 ± 9.63
	II	248.17 ± 18.74	249.00± 17.88	248.67± 17.81
	III	252.50± 10.31	267.17 ± 10.60	285.50 ± 8.94

Group I, II, III = horizontal mattress sutures, mattress sutures with collagen glue, and sole application of collagen glue, respectively. Means bearing different superscript within the row differ significantly between days ($p<0.05$).

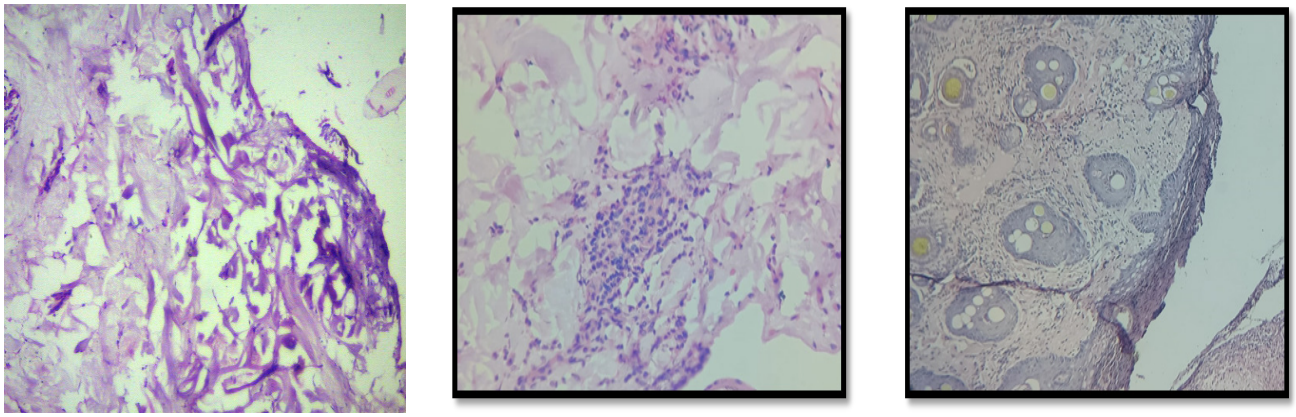
groups, indicating no altered response towards utility of collagen glue. Since, aural haematoma remained localized without any systemic involvement which further proves non-significant changes in haematological parameters. The total protein, albumin, globulin, albumin : globulin ratio and fibrinogen were found to be unaffected at all-time intervals and fluctuated around the normal physiological values in all the groups.

Histopathological observations (H&E staining) on day 3 revealed, presence of inflammatory cells and haemorrhage in group I (Fig. 4a), mild proliferation of fibroblast and least neovascularization in group II (Fig. 4b) and mild inflammatory cells, with active fibroblast proliferation and neovascularization in group III (Fig. 4c). On day 7, least fibroblast activity and minimal epithelialization was observed in group I (Fig. 5a), moderate fibroblast activity was observed



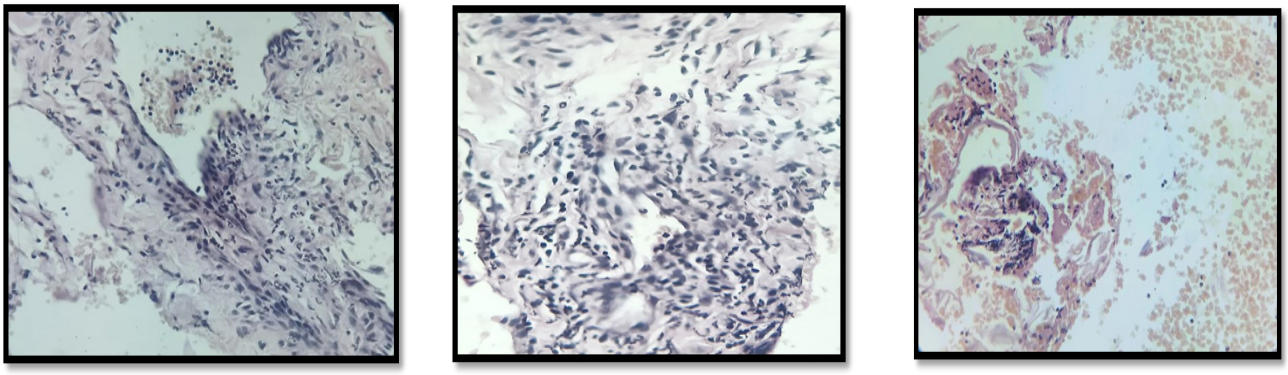
(a) (b) (c)

Fig. 4: Microphotograph of H&E stained sections on day 3rd of group I, II and III, respectively.



(a) (b) (c)

Fig. 5: Microphotograph of H&E stained sections on day 7th of group I, II and III, respectively.

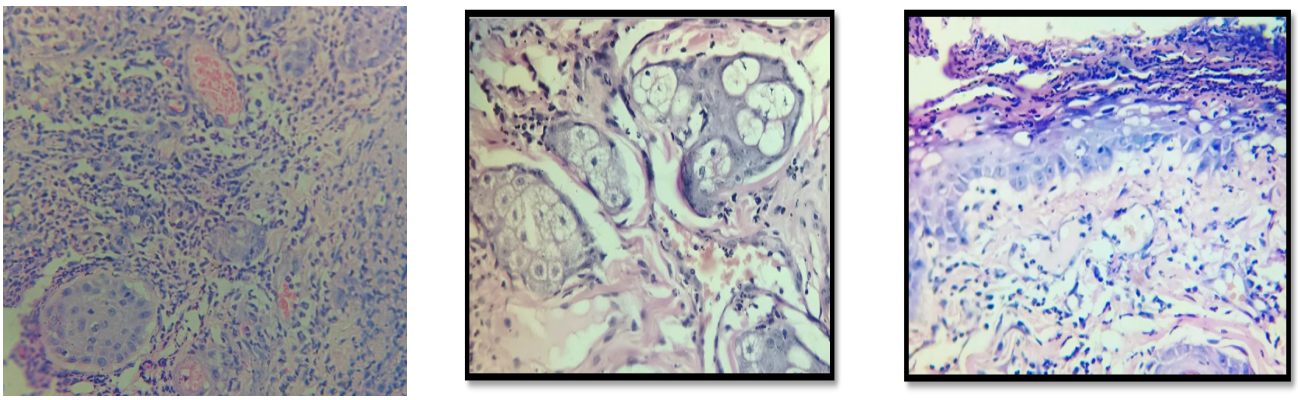


(a) (b) (c)

Fig. 6: Microphotograph of Van Gieson stained sections on day 3rd of group I, II and III, respectively.

in group II (Fig. 5b), while increased fibroblast proliferation, neovascularization and epithelisation was observed in group III (Fig. 5c) (Table 3). The inflammation was least in group III than group II and group I postoperatively, which suggests early progression of healing process. In group II and III, where collagen glue was utilized as a scaffold for tissue remodelling, repair and implant stabilization, fibroblast growth occurred quickly (Gokulakrishnan *et al.*, 2023). Sharma *et al.* (2022) also reported that the utilisation of collagen scaffolds facilitates early healing.

Histopathological observations by Van Gieson staining on day 3 revealed deficient collagen fibres density, arrangement and thickness in group I (Fig. 6a), less collagen fibre formation in group II (Fig. 6b), while presence of abundant collagen



(a) (b) (c)

Fig. 7: Microphotograph of Van Gieson stained sections on day 7th of group I, II and III, respectively.

Table 3: Mean ± SE of histopathological scoring for H&E staining and Van Gieson staining

Scoring system	Item	Group 1		Group 2		Group 3	
		Day 3	Day 7	Day 3	Day 7	Day 3	Day 7
H&E staining	Neutrophils	2.1^{Aa}±0.22	1.83^{Aa}±0.7	2.17^{Aa}±0.21	1.16^{Ab}±0.33	2.83^{Aa}±0.3	1.09^{Ab}±0.21
	Lymphocytes	2.34^{Aa}±02	2.50^{Aa}±0.22	2.43^{Aa}±0.22	2.60^{Aa} ± 0.21	2.89^{Aa}±0.31	3.33^{Ab}±0.21
	Macrophages	0.83^{Aa}±0.7	1.97^{Ab}±0.37	1.66^{Aa}±0.22	2.34^{Aa} ± 0.45	2.67^{Ba}±0.31	2.67^{Aa}±0.21
	Fibroblasts	1.67^{Aa}±0.1	2.34^{Aa}±0.21	2.12^{Aa}±0.31	2.43^{Aa} ± 0.26	2.67^{Ba}±0.12	3.33^{Ba}±0.22
	New vessels	2.17^{Aa}±0.1	2.23^{Aa}±0.21	2.28^{Aa}±0.31	2.62^{Aa} ± 0.26	2.67^{Aa}±0.14	2.78^{Ba}±0.22
Van Gieson staining	Collagen fibre density	1.33^{Aa}±0.21	1.67^{Aa}±0.21	1.83^{Aa}±0.21	2.33^{Ba}±0.42	2.44^{Ba}±0.22	3.20^{Cb}±0.22
	Collagen fibre arrangement	1.33^{Aa}±0.21	1.67^{Aa}±0.21	1.67^{Aa}±0.21	2.33^{Ab}±0.21	2.37^{Aa}±0.21	3.30^{Bb}±0.17
	Collagen fibre thickness	1.33^{Aa}±0.21	2.00^{Aa}±0.37	1.98^{Aa}±0.21	2.33^{Aa}±0.26	2.83^{Ba}±0.17	3.40^{Bb}±0.22

Means bearing different superscript differ significantly ($p<0.05$) within group (a,b) and between groups (A,B,C)

bundles at the incision site and intra auricular cartilage was in group III (Fig. 6c). On day 7, collagen fibres in the granulation tissue were few, thin, and less organized in group I (Fig. 7a), moderate collagen fibre density, thickness, and organization in group II (Fig. 7b), while the arrangement, density, and thickness of the collagen fibres were marked in group III (Fig. 7c)(Table 3). The early onset of epithelialization was observed in group III along with well-formed collagen and neovascularization than other groups. Balwada *et al*. (2014) stated that early epithelialization is the result of collagen infiltration.

Conclusion

From the findings of the current experiment it was concluded that, the bubaline derived composite collagen glue was proven to be effective, non-invasive, sutureless treatment modality for aural haematoma in dogs. It improved healing, minimized inflammation and resulted in superior cosmetic outcomes.

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