CHAPTER VI
SUMMARY AND CONCLUSIONS

6.1 Summary

Extended abdominal defect repair present an ongoing challenge to reconstructive surgery. Although several repair techniques have been described, synthetic materials are currently being investigated but failed to elicit biological cues similar to biological matrices. However, native biological materials tend to immunogenic and hence are acellularized to minimize their immunogenicity. Therefore, present clinical study was conducted with two objectives viz. 1. To optimize a procedure for preparation of caprine acellular dermal matrix (CADM), and 2. To evaluate biocompatibility of prepared matrix for abdominal hernioplasty in buffaloes.

Fresh skin of caprine origin (Capra hircus) was subjected to de-epithelialization and acellularization protocols. For de-epithelialization four protocols viz. sodium chloride (2 M) with trypsin (0.25 %), sodium chloride (2 M) with trypsin (0.5 %), sodium chloride (4 M) with trypsin (0.25 %), and sodium chloride (4 M) with trypsin (0.5 %) under constant agitation for 6 and 8 h at room temperature were tested. De-epithelialization completeness was confirmed microscopically. De-epithelialized cellular dermis was further subjected to acellularization protocols for making them acellular. Four protocols viz. 1 % sodium dodecyl sulphate (SDS), 2 % SDS, 3 % SDS, and 4 % SDS under constant agitation for 12, 24, 48, and 72 h at room temperature were tested. Acellularization completeness was confirmed histologically. Prepared caprine acellular dermal matrix (CADM) was further characterized by Masson’s trichrome and Weigert staining, scanning electron microscopy (SEM), DNA quantification, and Fourier transform infrared (FTIR) spectroscopy. Twenty Jafarabadi buffaloes (17 females and 3 males) weighing from 90 to 600 kg aged between 1 and 108 months with abdominal hernias were assigned into two equal groups: implanted with caprine acellularized dermal matrix [CADM (I) (average weight 159.5 kg) (average hernial ring size 42 cm$^2$)] and polypropylene mesh [PPM (II) (average weight 359.7 kg) (average hernial ring size 135.9 cm$^2$)]. Clinical, hematological, biochemical and circulating antioxidants evaluation was carried on days 0, 7 and 15 to assess healing progress.
Summary & Conclusions

Histologically, complete de-epithelialization of fresh caprine skin was observed following treatment with 0.25 % trypsin/4 M NaCl combination for 8 h under physical agitation at room temperature. Treatment of de-epithelialized cellular dermis with 2 % sodium dodecyl sulphate (SDS) for 48 h under physical agitation at room temperature revealed absence of cells and orderly arranged collagen fibres. Masson’s trichome (MTS) and Weigert’s resorcin fuchsin (WRF) staining of tissue section further confirmed intact collagen and elastic fibers within caprine acellular dermal matrix (CADM). SEM examination confirmed preservation of collagen fibrils arrangement within CADM. DNA concentration was 662.56 ± 156.11 ng/mg of tissue (280.20-920.25 ng/mg of tissue), 306.13 ± 52.68 ng/mg of tissue (130.10-393.50 ng/mg of tissue), and 46.20 ± 7.94 ng/mg of tissue (19.05-68.57 ng/mg of tissue) in NCS, DCS and CADM. DNA content was significantly ($P < 0.05$) decreased in CADM as compared to NCS, and nonsignificantly ($P > 0.05$) decreased in CADM as compared to DCS. A nonsignificant ($P > 0.05$) decrease in DNA content was also observed in DCS as compared to NCS. Treatment with 0.25 % trypsin/4 M NaCl combination for 8 h resulted in 53.80 % reduction in DNA content of the caprine skin. Treatment with 0.25 % trypsin/4 M NaCl combination for 8 h followed by 2 % SDS for 48 h resulted in 93.03 % reduction in DNA contents of the caprine skin. In FTIR analysis, amide A band was found at 3288.74 cm$^{-1}$ for native caprine skin (NCS), 3289.70 cm$^{-1}$ for de-epithelialized caprine skin (DCS), 3306.10 cm$^{-1}$ for CADM and 3285.85 cm$^{-1}$ for hydroxyproline standard. Amide B band is related to an asymmetrical stretch of CH$_2$ and was observed at 2936.72 cm$^{-1}$ for NCS, 2953.12 cm$^{-1}$ for DCS, 2953.12 cm$^{-1}$ for CADM, and 2951.19 cm$^{-1}$ for hydroxyproline standard. The amide I band associated with C=O stretching vibration or hydrogen bond coupled with COO was recorded at 1657.87 cm$^{-1}$ for NCS, 1658.84 cm$^{-1}$ for DCS, 1666.55 cm$^{-1}$ for CADM and 1640.51 cm$^{-1}$ for hydroxyproline standard. The amide II of NCS, DCS, CADM and hydroxyproline standard appeared at 1546.96 cm$^{-1}$, 1530.57 cm$^{-1}$, 1547.93 cm$^{-1}$, and 1548 cm$^{-1}$, respectively. The Amide II peak arises due to C-N stretching and N-H in plane bending from amide linkages, including wagging vibrations of CH$_2$ groups from the glycine backbone and proline side-chains. The amide III band was found at 1236.41 cm$^{-1}$ for NCS, 1238.34 cm$^{-1}$ for DCS, 1238.34 cm$^{-1}$ for CADM, and 1238.34 cm$^{-1}$ for hydroxyproline standard confirming presence of hydrogen bonds. Furthermore, the absorption peak at around 1400 cm$^{-1}$ was also
found in DCS and CADM, corresponded to vibration of pyrrolidine rings in proline and hydroxyproline.

All the animals of both the group remained slightly anorexic and dull for first 2 days after the operation. Food and water intake were normalized by the postoperative day 3. Swelling was recorded on postoperative day 1 in both groups, and thereafter swelling at the operated site continued to decrease gradually, and completely subsided on days 3 and 7 in CADM (I) and PPM (II) groups, respectively. During study period surgical site infection was found in 6 out of 10 cases in PPM (II), whereas none in CADM (I). In CADM (I) extreme swelling observed in one animal with discolouration of skin around operated site on day 7. On physical palpation hard, warm swelling found, without serous or pus exudate. It was gradually decreased on day 10. On day 15, normal epithelization was evident and healing take place. Recurrence noticed in this case after 45 days of operation. In other cases of group CADM (I) incision site healing was normal with healthy epithelization. In PPM (II) immediately after surgery mild surgical site haemorrhage observed in 2 animals. Postoperatively, fever, surgical site infection, purulent drainage, delay in healing observed in 5 animals of PPM (II). Hard, warm swelling noted on day 1 in all the animal and it gradually decreased on day 7. In one animal suture breakdown due to swelling and visualization of implanted mesh at surgical site was observed on day 7. This resulted in persistent sinus and pus discharge. Health status of animal was further deteriorated and died on postoperative day 20.

Hemoglobin (Hb) concentration in CADM (I), Hb concentration was 12.78 ± 0.21mg/dL (11.70-13.80 mg/dL), 12.59 ± 0.37mg/dL (11.00-14.10 mg/dL) and 13.07 ± 0.11mg/dL (12.20-13.40 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), Hb concentration was 11.96 ± 0.46mg/dL (9.50-14.90 mg/dL), 12.01 ± 0.49mg/dL (9.30-14.50 mg/dL) and 12.00 ± 0.39mg/dL (9.80-13.70 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), Hb concentration nonsignificantly (P > 0.05) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 15, Hb concentration was significantly (P < 0.05) higher in CADM (I) as compared to PPM (II), whereas remained nonsignificantly (P > 0.05) changed on day 7.

Packed cell volume (PCV) in CADM (I) was 38.32 ± 0.62 % (35.13-41.3 %), 36.04 ± 1.45 % (27.98-43.45 %) and 39.21 ± 0.35 % (36.45-40.34 %) on days 0, 7 and 15, respectively. In PPM (II), PCV was 35.87 ± 1.36 % (28.63-44.56 %), 40.41 ±
Summary & Conclusions

3.17 % (27.98-61.26 %) and 35.99 ± 1.16 % (29.49-41.22 %) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), PCV nonsignificantly changed (P > 0.05) on days 7 and 15 as compared to their respective day 0 value. On days 0 and 7, PCV was found nonsignificantly (P > 0.05) changed between CADM (I) and PPM (II) groups, whereas significantly (P < 0.05) higher in CADM (I) on day 15 in comparison to PPM (II).

Erythrocyte counts in CADM (I) was 12.90 ± 0.31 x10^6/µL (11.4-14.3 x10^6/µL), 12.83 ± 0.20 x10^6/µL (11.9-13.9 x10^6/µL) and 13.32 ± 0.15 x10^6/µL (12.6-14.1 x10^6/µL) on days 0, 7 and 15, respectively. In PPM (II), erythrocyte was 11.27 ± 0.58 x10^6/µL (8.7-14.0 x10^6/µL), 12.63 ± 1.38 x10^6/µL (3.2-18.9 x10^6/µL) and 12.51 ± 0.40 x10^6/µL (9.5-13.7 x10^6/µL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), erythrocyte count nonsignificantly (P > 0.05) changed on days 7 and 15 as compared to their respective day 0 value. On day 0, erythrocyte count was significantly (P < 0.05) higher in CADM (I), whereas nonsignificantly (P > 0.05) changed on days 7 and 15 in comparison to PPM (II).

Mean corpuscular volume (MCV) in CADM (I) was 39.0 ± 1.89 fL (35-55 fL), 45.0 ± 2.81 fL (35-60 fL) and 42.4 ± 2.30 fL (36-59 fL) on days 0, 7 and 15, respectively. In PPM (II), MCV was 48.4 ± 3.26 fL (36-63 fL), 47.2 ± 3.61 fL (37-66 fL) and 44.7 ± 3.28 fL (35-65 fL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), MCV nonsignificantly (P > 0.05) changed on days 7 and 15 as compared to their respective day 0 value. On day 0, MCV was significantly (P < 0.05) lower in CADM (I), whereas nonsignificantly (P > 0.05) changed on days 7 and 15 in comparison to PPM (II).

Mean corpuscular hemoglobin (MCH) in CADM (I) was 11.80 ± 0.36 pg (10.40-14.20 pg), 12.91 ± 0.47 pg (11.50-15.50 pg) and 13.20 ± 0.38 pg (11.90-15.30 pg) on days 0, 7 and 15, respectively. In PPM (II), MCH was 13.59 ± 0.75 pg (10.30-16.78 pg), 14.00 ± 0.49 pg (11.80-16.99 pg) and 13.37 ± 0.52 pg (11.60-16.40 pg) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), MCH nonsignificantly (P > 0.05) changed on days 7 and 15 as compared to their respective day 0 value. On day 0, MCH was significantly (P < 0.05) lower in CADM (I), whereas nonsignificantly (P > 0.05) changed on days 7 and 15 in comparison to PPM (II).

Mean corpuscular hemoglobin concentration (MCHC) in CADM (I) was 32.54 ± 1.36 g/dL (25.70-40.00 g/dL), 34.00 ± 1.42 g/dL (25.37-41.00 g/dL) and 34.56 ± 1.01 g/dL (31.30-39.90 g/dL) on days 0, 7 and 15, respectively. In PPM (II), MCHC
was 29.16± 1.28 g/dL (20.10-33.50 g/dL), 30.05 ± 1.48 g/dL (23.90-39.90 g/dL) and 30.42 ± 1.07 g/dL (25.30-36.20 g/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), MCHC nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 7, MCHC nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II), whereas significantly \((P < 0.05)\) higher on day 15 in CADM (I) in comparison to PPM (II).

Leukocyte counts in CADM (I) was 17.68 ± 2.24 x 10^3/µL (10.8-28.8 x 10^3/µL), 13.07 ± 1.44 x 10^3/µL (7.94-23.02 x 10^3/µL) and 12.94 ± 1.81 x 10^3/µL (8.78-20.21 x 10^3/µL) on days 0, 7 and 15, respectively. In PPM (II), leukocyte was 13.03 ± 0.79 x 10^3/µL (9.51-16.9 x 10^3/µL), 12.36 ± 1.75 x 10^3/µL (4.08-23.03 x 10^3/µL) and 16.25 ± 1.43 x 10^3/µL (6.7-22.12 x 10^3/µL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), leukocyte count nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7, 15, leukocyte count was nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II).

Monocytes counts in CADM (I) was 3.98 ± 0.31 % (2.2-5 %), 3.20 ± 0.39 % (1.1-5.5 %) and 2.56 ± 0.28 % (1-4.2 %) on days 0, 7 and 15, respectively. In PPM (II), monocytes count was 3.68±0.42 % (1.2-5.3 %), 3.17 ± 0.59 % (1-7 %) and 3.86 ± 0.40 % (2-6 %) on days 0, 7 and 15, respectively. In CADM (I), monocytes count significantly \((P < 0.05)\) lower on day 15 as compared today 0 value, however, nonsignificantly \((P > 0.05)\) changed on day 7 as compared todays 0 and 15 values. In PPM (II), monocytes count remained nonsignificantly \((P > 0.05)\) on days 7 and 15 as compared today 0 value. On days 0 and 7, monocytes count was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II). On day 15, significantly \((P < 0.05)\) lower monocytes count was recorded in CADM (I) as compared to PPM (II).

Neutrophils counts in CADM (I) was 42.08 ± 4.25 % (27.8-67.33 %), 28.38 ± 2.63 % (17.4-47.33 %) and 26.89 ± 1.50 % (16.5-34.02 %) on days 0, 7 and 15, respectively. In PPM (II), neutrophils count was 36.49±5.97 % (9.6-59.6 %), 39.04 ± 6.38 % (11-68 %) and 42.68 ± 3.51 % (24.55-66 %) on days 0, 7 and 15, respectively. In CADM (I), neutrophils counts significantly \((P < 0.05)\) lower on days 7 and 15 as compared today 0 value. In PPM (II), neutrophils count nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to day 0 value. On days 0 and 7, neutrophils count was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM.
On day 15, significantly ($P < 0.05$) lower neutrophils count was recorded in CADM (I) as compared to PPM (II).

Basophil counts in CADM (I) was $0.02 \pm 0.02\%$ (0.0-0.2 %), $0.02 \pm 0.02\%$ (0.0-0.2 %) and $0.00 \pm 0.00\%$ (0.0-0.0 %) on days 0, 7 and 15, respectively. In PPM (II), basophil count was $0.05 \pm 0.03\%$ (0.0-0.3 %), $0.03 \pm 0.03\%$ (0.0-0.3 %) and $0.00 \pm 0.00\%$ (0.0-0.0 %) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), basophil count remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, basophil count was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II).

Eosinophils counts in CADM (I) was $0.34 \pm 0.03\%$ (0.2-0.5 %), $0.50 \pm 0.08\%$ (0.1-0.8 %) and $0.38 \pm 0.07\%$ (0.1-0.8 %) on days 0, 7 and 15, respectively. In PPM (II), eosinophils count was $0.46 \pm 0.04\%$ (0.3-0.6 %), $0.46 \pm 0.07\%$ (0.1-0.7 %) and $0.44 \pm 0.08\%$ (0.1-0.9 %) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), eosinophils count remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to their respective day 0 value. On day 0, eosinophils count was found significantly ($P < 0.05$) lower in CADM (I) group as compared to PPM (II) group. However, on days 7 and 15, eosinophils count was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II).

Platelet counts in CADM (I) was $303.20 \pm 20.30 \times 10^3/\mu L$ (196-420 x $10^3/\mu L$), $368.20 \pm 37.76 \times 10^3/\mu L$ (260-657 x $10^3/\mu L$) and $339.40 \pm 21.30 \times 10^3/\mu L$ (204-458 x $10^3/\mu L$) on days 0, 7 and 15, respectively. In PPM (II), platelet count was $331.20 \pm 34.16 \times 10^3/\mu L$ (200-519 x $10^3/\mu L$), $325.00 \pm 18.70 \times 10^3/\mu L$ (250-438 x $10^3/\mu L$) and $350.00 \pm 29.94 \times 10^3/\mu L$ (239-538 x $10^3/\mu L$) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), platelet count remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, platelet count was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II) groups.

Erythrocyte sedimentation rate (ESR) in CADM (I) was $23.1 \pm 3.21$ mm/h (10-38 mm/h), $34.7 \pm 3.39$ mm/h (18-57 mm/h) and $32.6 \pm 3.96$ mm/h (14-56 mm/h) on days 0, 7 and 15, respectively. In PPM (II), ESR was $35.0 \pm 4.48$ mm/h (16-62 mm/h), $49.2 \pm 6.11$ mm/h (32-86 mm/h) and $60.7 \pm 8.70$ mm/h (32-117 mm/h) on days 0, 7 and 15. In CADM (I), ESR remained nonsignificantly ($P > 0.05$) on days 7 and 15 as compared to day 0 value. In PPM (II), ESR was significantly ($P < 0.05$)
changed higher on days 15 as compared to day 0 value, however remained nonsignificantly \((P > 0.05)\) changed on day 7 as compared to days 0 and 15 values. On days 0 and 15, ESR was significantly \((P < 0.05)\) lower in CADM (I) group as compared to PPM (II) group. On day 7, ESR was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II) groups.

Total protein (TP) in CADM (I) was \(6.06 \pm 0.06 \text{ g/L (5.67-6.29 g/L)}\), \(6.32 \pm 0.20 \text{ g/L (5.50-7.78 g/L)}\) and \(6.10 \pm 0.26 \text{ g/L (4.84-7.46 g/L)}\) on days 0, 7 and 15, respectively. In PPM (II), TP concentration was \(6.60 \pm 0.29 \text{ g/L (5.44-7.89 g/L)}\), \(7.04 \pm 0.33 \text{ g/L (5.15-8.10 g/L)}\) and \(7.05 \pm 0.30 \text{ g/L (5.41-8.03 g/L)}\) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), TP concentration remained nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 7, TP concentration was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II) group. However, on day 15, TP concentration was significantly \((P < 0.05)\) lower in CADM (I) as compared to PPM (II) group.

Albumin concentration in CADM (I) was \(2.67 \pm 0.06 \text{ g/L (2.37-2.95 g/L)}\), \(2.64 \pm 0.05 \text{ g/L (2.45-2.85 g/L)}\) and \(2.51 \pm 0.07 \text{ g/L (2.28-2.94 g/L)}\) on days 0, 7 and 15, respectively. In PPM (II), albumin concentration was \(2.57 \pm 0.03 \text{ g/L (2.41-2.70 g/L)}\), \(2.53 \pm 0.09 \text{ g/L (2.19-3.22 g/L)}\) and \(2.63 \pm 0.10 \text{ g/L (2.10-3.19 g/L)}\) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), albumin concentration remained nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, albumin concentration was found nonsignificant \((P > 0.05)\) between CADM (I) and PPM (II) group.

Globulin concentration in CADM (I) was \(3.39 \pm 0.09 \text{ g/L (2.90-3.81 g/L)}\), \(3.69 \pm 0.19 \text{ g/L (2.95-5.07 g/L)}\) and \(3.59 \pm 0.28 \text{ g/L (2.40-5.17 g/L)}\) on days 0, 7 and 15, respectively. In PPM (II), globulin concentration was \(4.03 \pm 0.29 \text{ g/L (2.78-5.28 g/L)}\), \(4.51 \pm 0.30 \text{ g/L (2.85-5.61 g/L)}\) and \(4.42 \pm 0.29 \text{ g/L (3.06-5.47 g/L)}\) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), globulin concentration remained nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, globulin concentration was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II) group.

Albumin-globulin (A:G) ratio in CADM (I) was \(0.80 \pm 0.0368 \text{ (0.65-0.96), 0.73 \pm 0.0360 \text{ (0.53-0.90) and 0.74 \pm 0.0620 \text{ (0.44-1.02) on days 0, 7 and 15, respectively. In PPM (II), A:G ratio was 0.67 \pm 0.051 \text{ (0.48-0.96), 0.58 \pm 0.043 \text{ (0.43-0.81) and 0.62 \pm 0.053 \text{ (0.44-0.91) on days 0, 7 and 15, respectively. In CADM (I)
and PPM (II), A:G ratio remained nonsignificantly \( (P > 0.05) \) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 15, A:G ratio was found nonsignificantly \( (P > 0.05) \) changed between CADM (I) and PPM (II) group. However, on day 7, A:G ratio was significantly \( (P < 0.05) \) higher in CADM (I) as compared to PPM (II) group.

Glucose concentration in CADM (I) was 94.00 ± 6.53 mg/dL (75-146 mg/dL), 63.00 ± 10.94 mg/dL (23-116 mg/dL) and 68.20 ± 11.08 mg/dL (18-70 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), glucose concentration was 92.10 ± 12.51 mg/dL (52-155 mg/dL), 95.80 ± 11.65 mg/dL (56-143 mg/dL) and 96.90 ± 10.26 mg/dL (45-142 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), glucose concentration remained nonsignificantly \( (P > 0.05) \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, glucose concentration was found nonsignificantly \( (P > 0.05) \) changed between CADM (I) and PPM (II) groups.

Creatinine concentration in CADM (I) was 1.45 ± 0.13 mg/dL (1.10-2.25 mg/dL), 1.36 ± 0.10 mg/dL (0.96-2.05 mg/dL) and 1.60 ± 0.16 mg/dL (1.05-2.45 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), creatinine concentration was 1.45 ± 0.11 mg/dL (0.91-2.04 mg/dL), 1.55 ± 0.08 mg/dL (1.24-2.0 mg/dL) and 1.65 ± 0.14 mg/dL (0.93-2.47 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), creatinine concentration remained nonsignificantly \( (P > 0.05) \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, creatinine concentration was found nonsignificantly \( (P > 0.05) \) changed between CADM (I) and PPM (II) group.

Urea concentration in CADM (I) was 42.30 ± 4.19 mg/dL (20.69-63.83 mg/dL), 50.13 ± 4.34 mg/dL (24-69.59 mg/dL) and 35.44 ± 5.00 mg/dL (17.03-70.36 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), urea concentration was 44.74 ± 6.13 mg/dL (10.86-71.11 mg/dL), 39.35 ± 4.67 mg/dL (10.44-68.42 mg/dL) and 46.52 ± 5.24 mg/dL (25.04-71.80 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), urea concentration remained nonsignificantly \( (P > 0.05) \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, urea concentration was found nonsignificantly \( (P > 0.05) \) changed between CADM (I) and PPM (II) group.

Urea nitrogen concentration in CADM (I) was 19.76 ± 1.96 mg/dL (9.67-29.83 mg/dL), 22.03 ± 2.80 mg/dL (3.68-32.52 mg/dL) and 16.57 ± 2.34 mg/dL
Summary & Conclusions

(7.96-32.88 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), urea nitrogen concentration was 20.91 ± 2.87 mg/dL (5.07-33.23 mg/dL), 18.39 ± 2.18 mg/dL (4.88-31.97 mg/dL) and 21.73 ± 2.45 mg/dL (11.70-33.55 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), urea nitrogen concentration remained nonsignificantly \( P > 0.05 \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, urea nitrogen concentration was found nonsignificantly \( P > 0.05 \) changed between CADM (I) and PPM (II) group.

Cholesterol concentration in CADM (I) was 153.4 ± 14.83 mg/dL (104-219 mg/dL), 157 ± 17.56 mg/dL (106-191 mg/dL) and 165.7 ± 22.06 mg/dL (67-256 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), cholesterol concentration was 157 ± 9.54 mg/dL (106-191 mg/dL), 140 ± 17.43 mg/dL (27-230 mg/dL) and 143.4 ± 18.36 mg/dL (74-212 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), cholesterol concentration remained nonsignificantly \( P > 0.05 \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, cholesterol concentration was found nonsignificantly \( P > 0.05 \) changed between CADM (I) and PPM (II) group.

Triglyceride concentration in CADM (I) was 37.9 ± 5.63 mg/dL (16-82 mg/dL), 40.6 ± 6.21 mg/dL (16-82 mg/dL) and 36.4 ± 8.06 mg/dL (14-83 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), triglyceride concentration was 31.5 ± 9.54 mg/dL (16-51 mg/dL), 30.6 ± 5.17 mg/dL (13-62 mg/dL) and 38.6 ± 5.79 mg/dL (8-64 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), triglyceride concentration remained nonsignificantly \( P > 0.05 \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, triglyceride concentration was found nonsignificantly \( P > 0.05 \) changed between CADM (I) and PPM (II) group.

High-density lipoprotein (HDL) in CADM (I) was 49.8 ± 4.42 mg/dL (31.86-68.61 mg/dL), 47.38 ± 5.65 mg/dL (14.04-76.1 mg/dL) and 49.52 ± 5.92 mg/dL (20.55-72.71 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), HDL concentration was 49.04 ± 2.18 mg/dL (39.15-56.99 mg/dL), 39.49 ± 4.54 mg/dL (10.43-64.24 mg/dL) and 70.32 ± 28.44 mg/dL (23.26-321.69 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), HDL concentration remained nonsignificantly \( P > 0.05 \) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, HDL concentration was found nonsignificantly \( P > 0.05 \) changed between CADM (I) and PPM (II) group.
Low-density lipoprotein (LDL) in CADM (I) was 31.64 ± 3.10 mg/dL (18.34-45.82 mg/dL), 29.88 ± 3.30 mg/dL (9.31-41.97 mg/dL) and 34.43 ± 4.52 mg/dL (15.03-52.86 mg/dL) on days 0, 7 and 15, respectively. In PPM (II), LDL concentration was 34.98 ± 3.12 mg/dL (20.5-49.22 mg/dL), 31.82 ± 4.05 mg/dL (6.56-51.16 mg/dL) and 30.15 ± 3.97 mg/dL (16.14-46.23 mg/dL) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), LDL concentration remained nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, LDL concentration was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II) group.

Alkaline phosphatase (ALP) in CADM (I) was 214.64 ± 26.51 IU/L (120-380.2 IU/L), 203.42 ± 34.94 IU/L (53.2-404 IU/L) and 167.84 ± 23.32 IU/L (84.5-318 IU/L) on days 0, 7 and 15, respectively. In PPM (II), ALP activity was 145.76 ± 24.03 IU/L (37-294 IU/L), 97.23 ± 20.44 IU/L (37.2-211.4 IU/L) and 130.82 ± 37.29 IU/L (26.2-424.2 IU/L) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), ALP activity remained nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, ALP activity was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II) group.

Lactate dehydrogenase (LDH) in CADM (I) was 1124.92 ± 41.92 IU/L (374.91-1517.65 IU/L), 1288.82 ± 56.63 IU/L (107.88-1399.93 IU/L) and 1125.38 ± 55.74 IU/L (257-454.65 IU/L) on days 0, 7 and 15, respectively. In PPM (II), LDH activity was 1276.29 ± 161.59 IU/L (189.52-1884.50 IU/L), 1270.13 ± 186.68 IU/L (77.03-1907.20 IU/L) and 1560.14 ± 229.49 IU/L (246.52-1969.59 IU/L) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), LDH activity remained nonsignificantly \((P > 0.05)\) changed on days 7 and 15 as compared to their respective day 0 value. Similarly, on days 0, 7 and 15, LDH activity was found nonsignificantly \((P > 0.05)\) changed between CADM (I) and PPM (II) group.

Creatine kinase (CK) in CADM (I) was 800.21 ± 110.86 IU/L (374.91-1517.65 IU/L), 645.61 ± 135.59 IU/L (107.88-1399.93 IU/L) and 343.24 ± 20.39 IU/L (257-454.65 IU/L) on days 0, 7 and 15, respectively. In PPM (II), CK activity was 807.48 ± 192.03 IU/L (189.52-1884.5 IU/L), 581.83 ± 168.11 IU/L (77.03-1907.2 IU/L) and 705.54 ± 208.77 IU/L (246.52-1969.59 IU/L) on days 0, 7 and 15, respectively. In CADM (I), CK activity was significantly \((P < 0.05)\) lower on day 15 as compared today 0 value, whereas, remain nonsignificantly \((P > 0.05)\) changed on day 7 as compared todays 0 and 15 value. In PPM (II), CK activity remained
nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to today 0 value. Similarly, on days 0, 7 and 15, CK activity was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II) group.

Alanine aminotransferase (ALT/SGPT) in CADM (I) was $38.43 \pm 3.81$ IU/L (21.29-56.76 IU/L), $41.77 \pm 5.13$ IU/L (10.87-62.27 IU/L) and $33.56 \pm 3.52$ IU/L (15.16-47.23 IU/L) on days 0, 7 and 15, respectively. In PPM (II), ALT activity was $45.3 \pm 6.38$ IU/L (18.56-91.82 IU/L), $38.61 \pm 7.89$ IU/L (10.11-101.83 IU/L) and $55.69 \pm 9.80$ IU/L (2.95-104.71 IU/L) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), ALT activity remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 7, ALT activity was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II) groups. However, on day 15, ALT activity was significantly ($P < 0.05$) lower in CADM (I) as compared to PPM (II) group.

Aspartate aminotransferase (AST/SGOT) in CADM (I) was $132.18 \pm 2.39$ IU/L (117.25-142.66 IU/L), $160.32 \pm 9.60$ IU/L (124.6-209.8 IU/L) and $123.16 \pm 7.59$ IU/L (87.63-162.57 IU/L) on days 0, 7 and 15, respectively. In PPM (II), AST activity was $150.05 \pm 10.84$ IU/L (120.77-238.69 IU/L), $163.04 \pm 17.85$ IU/L (98.46-305.16 IU/L) and $184.89 \pm 32.56$ IU/L (80.51-311.36 IU/L) on days 0, 7 and 15, respectively. In CADM (I), AST activity was significantly ($P < 0.05$) lower on day 15 as compared to days 0 and 7 value. In PPM (II), AST activity remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared today 0 value. On days 0 and 7, AST activity was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II) groups. However, on day 15, AST activity was significantly ($P < 0.05$) lower in CADM (I) as compared to PPM (II) group.

Gamma-glutamyl transferase (GGT) in CADM (I) was $4.86 \pm 0.80$ IU/L (117.25-142.66 IU/L), $4.75 \pm 0.58$ IU/L (124.6-209.8 IU/L) and $4.28 \pm 0.57$ IU/L (87.63-162.57 IU/L) on days 0, 7 and 15, respectively. In PPM (II), GGT activity was $7.18 \pm 1.05$ IU/L (120.77-238.69 IU/L), $5.67 \pm 0.82$ IU/L (98.46-305.16 IU/L) and $7.06 \pm 0.89$ IU/L (80.51-311.36 IU/L) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), GGT activity remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 7, GGT activity was found nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II) groups. However, on day 15, GGT activity was significantly ($P < 0.05$) lower in CADM (I) as compared to PPM (II) group.
Reduced glutathione (GSH) activity in CADM (I) was $6.78 \pm 0.50$ mmol/L (2.99-8.44 mmol/L), $7.07 \pm 0.54$ mmol/L (4.96-10.46 mmol/L) and $5.64 \pm 0.71$ mmol/L (2.87-10.19 mmol/L) on days 0, 7 and 15, respectively. In PPM (II), GSH activity was $4.59 \pm 0.53$ mmol/L (2.24-6.69 mmol/L), $4.71 \pm 0.64$ mmol/L (2.63-8.66 mmol/L) and $6.27 \pm 1.09$ mmol/L (3.07-13.02 mmol/L) on days 0, 7 and 15, respectively. In CADM (I) and PPM (II), GSH activity remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared to their respective day 0 value. On days 0 and 7, GSH activity was found significantly ($P < 0.05$) higher in CADM (I) as compared to PPM (II). However, on day 15, GSH activity remained nonsignificantly ($P > 0.05$) changed between CADM (I) and PPM (II) groups.

Catalase (CAT) activity in CADM (I) was $1.40 \pm 0.21$ U/mL (0.58-2.52 U/mL), $1.82 \pm 0.33$ U/mL (0.98-4.39 U/mL) and $2.57 \pm 0.30$ U/mL (1.32-3.97 U/mL) on days 0, 7 and 15, respectively. In PPM (II), CAT activity was $3.89 \pm 0.48$ U/mL (1.47-6.15 U/mL), $3.83 \pm 0.43$ U/mL (1.68-6 U/mL) and $4.39 \pm 0.71$ U/mL (1.46-7.22 U/mL) on days 0, 7 and 15, respectively. In CADM (I), CAT activity was significantly ($P < 0.05$) higher on day 15 as compared today 0 value, whereas, remained nonsignificantly ($P > 0.05$) changed on day 7 as compared to days 0 and 15 value. In PPM (II), CAT activity remained nonsignificantly ($P > 0.05$) changed on days 7 and 15 as compared today 0 value. On days 0, 7 and 15, CAT activity was found significantly ($P < 0.05$) lower in CADM (I) as compared to PPM (II).

6.2 Conclusions

1. The protocol for acellularization of caprine skin was optimized.
2. Treatment with 0.25 % trypsin/4 M NaCl combination for 8 h and 2 % SDS for 48 h are best for complete removal of cells, dermal adnexa and other skin structures of caprine skin.
3. Prepared caprine acellular dermal matrix is composed of 3-D collagen lattice as evident microscopically following special staining and by Fourier transform infrared spectroscopy.
4. Treatment with 0.25 % trypsin/4 M NaCl combination for 8 h and 2 % SDS for 48 h resulted in 93.03 % reduction in DNA content from fresh skin of caprine origin.
5. Caprine acellular dermal matrix is found biocompatible for abdominal hernioplasty in buffaloes as evident by clinical outcome.
6. Studies have demonstrated mesh infection, fistulae formation, and mesh extrusion in animals of PPM (II) group.